Sufentanil transfer in the human placenta during *in vitro* perfusion

B. Roy Krishna PhD,
Mark I. Zakowski MD,*
Gilbert J. Grant MD

Purpose: Sufentanil, a lipophilic opioid, is used to provide analgesia for labour and Caesarean section, but may cause neonatal depression. Factors affecting placental transfer of sufentanil were investigated using human placentas.

Study design: Transfer and uptake of sufentanil by the human placenta were studied using a single pass (open) *in vitro* perfusion model. The effects of change in sufentanil concentration (1–100 ng·mL⁻¹) and change in fetal pH (range 7.4–6.8) on placental transfer were studied. Placental metabolism of sufentanil and the effects of maternal protein content (fresh human plasma, albumin 4%, Media 199) on placental transfer were also investigated utilizing a closed (recirculated) *in vitro* perfusion system.

Results: Sufentanil transfer was 2% at five minutes and plateaued at 12% by 45 min. The clearance index (CI=sufentanil clearance/antipyrine clearance) was 0.56 ± 0.2 for maternal to fetal (MTF) and 0.44 ± 0.2 in the fetal to maternal (FTM) directions ($P=NS$). The CI was 0.5 ± 0.2 for 1 ng·mL⁻¹ and 0.61 ± 0.3 for 100 ng·mL⁻¹ sufentanil concentration ($P=NS$). The placenta contained 7.1 ± 2 and 9.8 ± 3 ng·g⁻¹ sufentanil following MTF and FTM perfusions for 90 min at 1 ng·mL⁻¹. The placenta did not metabolize sufentanil. After one hour MTF washout, placental sufentanil content was $2.3 \pm .5$ ng·g⁻¹ with 0.08 ng·mL⁻¹ sufentanil in the umbilical vein. Maternal plasma decreased MTF CI from 0.41 ± 0.1 for albumin and 0.4 ± 0.1 for Media 199 to $0.17 \pm .06$ for plasma ($P < 0.05$). Decreasing fetal pH increased MTF CI from $0.57 \pm .13$ at pH 7.4 to $1.6 \pm .4$ at pH 6.8 ($P < 0.05$).

Conclusion: Sufentanil crossed the placenta by passive diffusion and accumulated in placental tissue, which acted as a drug depot, slowing the initial transfer. Placental transfer was decreased by maternal plasma proteins, but not by albumin. Fetal acidosis increased placental transfer. Due to its low initial umbilical vein concentration, sufentanil may be the opioid of choice when delivery is imminent (<45 min).

Objectif : Le sufentanil, un morphinique utilisé pour procurer l'analgésie pendant le travail et l'accouchement par césarienne, peut causer une dépression chez le nouveau-né. Cette étude réalisée sur placentas humains s'intéresse aux facteurs qui influencent le transfert placentaire du sufentanil.

Type d'étude : Un modèle *in vitro* (ouvert) de perfusion à un passage a servi à l'étude du transfert et du captage du sufentanil par le plasma humain. On a recherché les effets du changement de la concentration du sufentanil (1–100 ng·mL⁻¹) et du pH foetal (écart de 7,4 à 6,8) sur le transfert placentaire. Le métabolisme placentaire du sufentanil et l'influence de la concentration maternelle en protéines (plasma humain frais, albumine 4%, Media 199) ont aussi été étudiés à l'aide d'un système de perfusion *in vitro* fermé.

Résultats : Le transfert du sufentanil était de 2% après cinq minutes et a atteint un plateau de 12 % après 45 min. L'index de clairance (IC = clairance du sufentanil/clairance de l'antipyrine) était $0,56 \pm 0,2$ en direction materno-foetale (MF) et de $0,44 \pm 0,2$ en direction foeto-maternelle (FM) ($P = NS$). L'IC était $0,5 \pm 0,2$ et $0,61 \pm 0,3$ pour des concentrations de sufentanil de 1 ng·mL⁻¹ et de 100 ng·mL⁻¹. Le placenta contenait $7,1 \pm 2$ et $9,8 \pm 3$ ng·g⁻¹ de sufentanil après des perfusions MF et FM de 90 min à 1 ng·mL⁻¹. Le placenta n'a pas métabolisé le sufentanil. Après une heure de rinçage MF, le contenu placentaire de sufentanil était $2,3 \pm 0,5$ ng·g⁻¹ dont 0,08 ng·mL⁻¹ dans la veine ombilicale. Le plasma maternel a diminué l'IC MF de $0,41 \pm 0,1$ pour l'albumine et de $0,4 \pm 0,1$ pour le Media 199 à $0,17 \pm 0,06$ pour le plasma ($P < 0,05$). La baisse du pH foetal a fait augmenter l'IC MF de $0,57 \pm 0,13$ au pH 7,4 à $1,6 \pm 0,4$ au pH 6,8 ($P < 0,05$).

Conclusion : Le sufentanil traverse le placenta par diffusion passive et s'accumule dans le tissu placentaire qui agit comme un réservoir et ralentit ainsi le transfert initial. Les protéines plasmatiques maternelles réduisent le transfert placentaire mais non l'albumine. L'acidose foetale accentue le transfert placentaire. À cause de sa faible concentration ombilicale veineuse initiale, le sufentanil pourrait s'avérer le morphinique de choix lorsque l'accouchement est imminent (< 45 min).

From the Department of Anesthesiology, New York University Medical Center, 560 First Avenue, New York, NY, 10016 and *The Department of Anesthesiology, Cedars-Sinai Medical Center, 8700 Beverly Blvd, Los Angeles, CA 90048.

This research was supported in part by the Foundation for Anesthesia Education and Research with a grant from Malinckrodt Anesthesiology.

Address correspondence to: Mark Zakowski MD; Phone: 310-855-5841; Fax: 310-854-0226; E-mail: ZakowskiM@CSMC.com

Accepted for publication May 30, 1997.

SUFENTANIL is a potent μ receptor agonist, which has been used as the sole agent for epidural and intrathecal labour analgesia,¹⁻³ in combination with low concentrations of bupivacaine for epidural labour analgesia,⁴⁻⁶ and as an analgesic supplement for Caesarean section.⁷ Infusions of epidural sufentanil in combination with local anaesthetics have become a popular technique for labour analgesia.^{4,8}

However, maternally administered opioids may cross the placenta and adversely affect the neonate.^{6,7} Placental transfer of opioids has been studied in animals and in humans.^{4,9,10} The very high lipid solubility of sufentanil should enhance its placental transfer.^{8,11} However, sufentanil is also highly bound to plasma proteins, potentially decreasing its availability for placental transfer. While studies have investigated the fetal/maternal ratio of sufentanil at the time of delivery,^{4,8} we have found little data examining the factors affecting sufentanil transfer and uptake in the human placenta. Knowledge of sufentanil placental transfer characteristics may help improve neonatal and maternal safety. This study examined the placental uptake, metabolism and rate of transfer of sufentanil as well as the effect of maternal plasma proteins and decreased fetal pH on sufentanil transfer across the human placenta during *in vitro* perfusion.

Methods

Following approval by the Institutional Review Board, placentas were obtained from healthy parturients after Caesarean section or vaginal delivery. Independent maternal and fetal circulations were established using a previously described *in vitro* perfusion model.^{12,13} Briefly, an intact placental lobule was selected and a tributary fetal artery and vein pair were cannulated and perfused. The lobule was mounted in a special chamber maintained at 37°C. Four blunt cannulae were inserted into the decidual plate and intervillous space to establish an independent maternal circulation. Perfusion pressures were measured by a pressure transducer (Cobe, Lakewood CO) and pressure monitor (PhysioControl VSM-1, Redmond WA). The perfusate was a standard solution of Media 199 with Earls' salts, adjusted to a pH of 7.40 using NaHCO₃ and equilibrated against O₂:CO₂ 95%:5%. To increase oncotic pressure, perfusion media contained dextran 40,000 MW at 3.5 g·L⁻¹ and 15 g·L⁻¹ with flow rates of 12 and 6 ml·min⁻¹ in the maternal and fetal circuits respectively. Antipyrine, a rapidly diffusible, flow-limited marker, and creatinine, a slowly diffused, membrane-limited reference compound, served to assess adequacy of perfusion. A 45 min stabilization period was allowed. Preliminary experiments showed that sufentanil bound to standard PVC tubing. Thus, the perfusion system contained only polyethylene lined

tubing (IMED, San Diego, CA), which did not bind sufentanil. All samples were handled in glass to avoid sufentanil binding to plastic. Preliminary experiments showed that the placental transfer of sufentanil was unaffected by the presence of fentanyl 1 ng·ml⁻¹ in the maternal perfusate. Thus, if the parturient received epidural fentanyl prior to delivery, there would be no interference with sufentanil.

The experimental protocol was designed to determine the rate and possible mechanisms of sufentanil transfer. Two sets of experiments were conducted: 1) maternal to fetal transfer (MTF) and 2) fetal to maternal transfer (FTM). The experiments used an open:open (single pass, no recirculation) design to study sufentanil transfer. In the MTF experiments (n=10), the maternal perfusate contained [³H]-sufentanil 1 ng·ml⁻¹, antipyrine and creatinine 100 mg·L⁻¹ for 90 min. After completion of sufentanil perfusion, placentas were assayed for sufentanil content (n=3) or were perfused without sufentanil for one hour (washout, n=3), and then assayed for sufentanil content. After perfusion without sufentanil for one hour (washout), other MTF placentas (n=4) were perfused MTF for 90 min using high dose sufentanil (100 ng·ml⁻¹) in the maternal solution to assess saturability of placental transfer.

In the FTM experiments (n=10) the fetal perfusate contained [³H]-sufentanil 1 ng·ml⁻¹, antipyrine and creatinine 100 mg·L⁻¹ for 90 min. After completion of sufentanil perfusion, placentas were assayed for sufentanil content (n=5) or were perfused without sufentanil for one hour (washout, n=5), and then assayed for sufentanil content.

Placental metabolism

Placental metabolism of sufentanil was investigated using a closed:closed (recirculated maternal and fetal circuits) design. Following 30 min stabilization, maternal and fetal circuits were closed, with 100 ml in each circuit. Maternal perfusate contained 10 μ g·ml⁻¹ sufentanil (n=5). Maternal and fetal samples were obtained every 30 min for three hours. Samples were extracted using NaOH and heptane/isoamyl alcohol as previously described.¹⁴ All samples were analyzed using high performance liquid chromatograph (Waters Nova-Pak CN column, Millipore, Philadelphia, PA).¹⁵ Sufentanil, desmethylsufentanil and norsufentanil standards (Janssen Research Labs, Belgium) were separated on HPLC with a range of 0.05–10 μ g·ml⁻¹ with a coefficient of variation (CV) of 8% and served as metabolite controls.

Protein binding

The effect of protein binding on placental transfer of sufentanil was determined using a closed:closed (recirculated) design (n=9) with 100 ml reservoir in maternal and fetal circuits. Maternal perfusate contained 10 ng·ml⁻¹

sufentanil, antipyrine and creatinine ($100 \text{ mg}\cdot\text{L}^{-1}$). Each experiment consisted of three 45 min phases: 1) Maternal perfusate consisting of Media 199, 2) Media 199 with 4% human albumin, and 3) Fresh pooled non-pregnant human plasma. The order of the three phases was rotated in each individual experiment. Fetal perfusate was Media 199 during all phases.

Protein binding of sufentanil was measured by centrifugal ultrafiltration dialysis using the Centrifree Micropartition system (Amicon, Beverly MA). All venous plasma samples were obtained from healthy, non-pregnant volunteers. All samples contained [^3H]-sufentanil $10 \text{ ng}\cdot\text{ml}^{-1}$. Percent binding was calculated.

pH experiment

The effect of changes in fetal pH on maternal to fetal sufentanil transfer was examined by changing the fetal Media 199 perfusate pH successively from 7.4, to 7.2, 7.0, 6.8 and returning to baseline 7.4 ($n=4$). Fetal pH was changed by adding HCl and increasing the PCO_2 to maintain the desired pH. Maternal perfusate was kept at constant pH 7.4, PCO_2 40 mmHg. Perfusate pH, PCO_2 and PO_2 were measured by a blood gas analyzer (Nova Stat 5, Waltham, MA).

Placental tissue was analyzed for sufentanil and perfusion media were analyzed for sufentanil, antipyrine and creatinine. Villous tissue was dissected from the perfused placenta and 1 g samples were dissolved in 1M NaOH/sodium dilauryl sulfate 0.2%. Sufentanil concentrations were determined by measuring counts per minute of [^3H]-sufentanil in a liquid scintillation counter and comparing with the known concentration of sufentanil per counts per minute (range 0.01 – $1 \text{ ng}\cdot\text{ml}^{-1}$, CV 9%) (Beckman Instrument LS-6000IC, Fullerton, CA). Antipyrine (range 5 – $100 \text{ mg}\cdot\text{L}^{-1}$, CV 7%) and creatinine (range 2 – $100 \text{ mg}\cdot\text{L}^{-1}$, CV 6%) were measured using colorimetric assays.^{13,16,17} The placental clearances of sufentanil, antipyrine and creatinine were calculated using standard formulas.^{12,13} The clearance index (sufentanil clearance/antipyrine clearance) and transfer index (sufentanil clearance/creatinine clearance) were used for interplacental comparisons and to provide insight into possible mechanisms of transfer.

Results were expressed as mean $\pm$ S.D. and results were analyzed using independent t test between placentas (MTF *vs* FTM) and dependent t test for studies within the same placenta (MTF low dose *vs* high dose). Repeated measures analysis of variance was used for the protein binding and pH experiments. A *P* value <0.05 was considered significant.

Results

During MTF transfer, umbilical vein sufentanil concentration was 2% of maternal concentration by five minutes and plateaued between 35–60 min (Figure 1).

TABLE I Sufentanil transfer indices

	Maternal to Fetal	Fetal to Maternal	<i>P</i>	<i>n</i>
sufentanil clearance ($\text{ml}\cdot\text{min}^{-1}$)	0.78 ± 0.3	1.0 ± 0.4	N.S.	10
clearance index	0.56 ± 0.2	0.44 ± 0.2	N.S.	10
transfer index	1.6 ± 0.4	1.7 ± 0.5	N.S.	10
placental tissue (sufentanil $\text{ng}\cdot\text{g}^{-1}$), after 90 min perfusion with sufentanil $1 \text{ ng}\cdot\text{ml}^{-1}$	7.1 ± 2	9.8 ± 3	N.S.	5
placental tissue (sufentanil $\text{ng}\cdot\text{g}^{-1}$), after 60 min drug washout	2.3 ± 0.5	3.5 ± 1	N.S.	5

At completion of the sufentanil infusion, the placental sufentanil tissue content was higher than the perfusate sufentanil concentration (Table I). During the washout phase of placental perfusion, the umbilical vein sufentanil concentration remained elevated at $0.08 \text{ ng}\cdot\text{ml}^{-1}$ after 60 min. After one hour washout, the placental sufentanil content was $2.31 \pm 0.5 \text{ ng}\cdot\text{g}^{-1}$ tissue, lower than after perfusion with sufentanil ($P < 0.05$). The MTF clearance, clearance index, and transfer index following $1 \text{ ng}\cdot\text{ml}^{-1}$ sufentanil (0.77 ± 0.3 , 0.5 ± 0.2 and 1.6 ± 0.5 , respectively) and $100 \text{ ng}\cdot\text{ml}^{-1}$ sufentanil (0.85 ± 0.3 , 0.61 ± 0.3 and 1.8 ± 0.6 , respectively) were not different.

During FTM transfer, maternal effluent sufentanil concentration also appeared in low concentrations (1%) by five min and plateaued between 45–80 minutes (Figure 1). Placental sufentanil tissue content was approximately ten fold higher than perfusate sufentanil concentration, although not different from that

FIGURE 1 Sufentanil transfer during placental perfusion with sufentanil $1 \text{ ng}\cdot\text{ml}^{-1}$. Maternal-to-fetal graph shows umbilical vein concentration, ($n=10$). Fetal-to-maternal graph shows maternal effluent concentration, ($n=10$).

Results are mean $\pm$ S.E.M. Note that maternal flow is twice that of fetal flow.

following MTF perfusion ($P = \text{NS}$, Table I). The maternal effluent sufentanil concentration remained elevated at $0.06 \text{ ng}\cdot\text{ml}^{-1}$ at 60 min of washout phase. Placental tissue values decreased to $3.5 \pm 1 \text{ ng}\cdot\text{g}^{-1}$ tissue after one hour of washout ($P < 0.05$), but was not different from that after MTF washout. Clearance values were similar for both MTF and FTM directions (Table I). Neither maternal nor fetal samples contained a measurable amount of the sufentanil metabolites desmethysufentanil or norsufentanil in the three hour metabolism experiments.

Sufentanil did not bind to dextran in the centrifugal ultrafiltration dialysis experiment. Protein binding of sufentanil to Media 199 with albumin 4%, and fresh human plasma were $74.2 \pm 4.7\%$, and $97 \pm 1.3\%$ ($n=10$) respectively. The MTF transfer of sufentanil was reduced when the maternal circulation was perfused with plasma compared with Media 199 and Media 199 with albumin 4% (Figure 2).

Decreasing the fetal perfusate pH from 7.4 to 6.8 resulted in increasing the maternal to fetal transfer by almost three fold (Figure 3). Clearance indices returned to baseline during perfusion at fetal pH 7.4.

Discussion

Our study suggests that the mechanism of placental sufentanil transfer is by passive diffusion. This is supported by the non-saturability of clearance indices over a 100 fold range and the equal bidirectional transfer. If active or facilitated transport existed, the mechanism would be saturable, resulting in a decreased equilibrium transfer rate at high concentrations. Indeed, drugs used to produce anaesthesia have been noted to cross the placenta by passive diffusion.¹¹ Sufentanil clearance is less than that for antipyrine and greater than that for creatinine and indicates that sufentanil was neither a flow limited nor a membrane limited drug.

Sufentanil crossed the placenta rapidly, but with a very low initial concentration. The high placental tissue uptake of sufentanil delayed the time to maximal umbilical vein concentration by 15–30 min compared with alfentanil.¹³ Although sufentanil is 20% non-ionized at pH 7.4 ($\text{pK}_a=8.0$) and is highly protein bound (93%), it crosses biological membranes quickly due to its extreme lipid solubility (octanol:water partition coefficient of 1757).^{11,18} However, sufentanil concentration in the fetal effluent was only 2% of maternal concentration at five minutes. Alfentanil, which has similar protein binding (92%) but is less lipophilic, reached 14% of maternal concentration at five minutes in a similar study.¹³

The high placental uptake of sufentanil was consistent with sufentanil protein binding and lipophilic

FIGURE 2 Effect of maternal proteins on maternal to fetal transfer of sufentanil. Media 199, fresh human plasma and Media 199 with 4% albumin were maternal perfusates in rotating order, ($n=9$).

Fetal perfusate was Media 199 throughout.

$P < 0.05$.

FIGURE 3 Effect of change in fetal perfusate pH on sufentanil clearance index, ($n=4$). Maternal perfusate held constant at pH 7.4.

* $P < 0.05$ compared with baseline pH 7.4.

characteristics. Sufentanil has a moderate distribution volume ($1.7 \text{ L}\cdot\text{kg}^{-1}$) and elimination half life (2.7 hr).¹⁸ The persistence of sufentanil in perfusate following one hour washout and the high sufentanil placental tissue levels shows that the placenta acts as a drug depot. In contrast, fentanyl placental tissue concentration was only three-fold higher than perfusate concentration during a similar study.¹⁹ Placental passage of sufentanil was less than that of fentanyl, which has an octanol:water partition coefficient half that of sufentanil.^{8,9,18,20} Alfentanil, which has a high protein

binding (92%), 7% of the lipid solubility of sufentanil and a much smaller volume of distribution, had a placental tissue content equal to that of perfusate.^{13,18} Thus, lipid solubility had a greater influence on placental tissue uptake than protein binding. The presence of a placental depot should slow equilibration of sufentanil to the fetus during increases or decreases of maternal plasma sufentanil concentration.

Thus, sufentanil use up to 45 min before delivery should result in less transfer than another opioid that the placenta does not bind, such as alfentanil. Neonates have shown signs of depression from sufentanil only at high doses, while other studies have shown sufentanil to result in less neonatal exposure than fentanyl.^{6-8,20} Indeed, in a sheep model, sufentanil was not detected in fetal arterial samples and the authors suggested that sufentanil may not cross the placenta as easily as other opioids.⁹ For dose-delivery time intervals greater than 45 minutes, the placenta may act as a drug depot and continue to supply sufentanil to the fetus even when the maternal blood level is decreasing. Thus, sufentanil may be the opioid of choice with respect to imminent (<45 min) neonatal exposure and depression.

pH effect

A reduction in the fetal perfusate pH to 6.8 increased MTF transfer almost three fold. The large increase in drug transfer is presumably due to an increase in the ionized form of the drug on the fetal side of the circulation, termed "ion trapping." The non-ionized form of drugs crosses biological membranes most easily, whereas the ionized form of the drug is relatively impermeable. Local anaesthetics are weak bases and have been shown to be "ion trapped," increasing total drug concentration in the acidotic fetal circulation.^{11,21,22} The opioids are also weak bases and, thus, ion trapping would be expected to occur. Decreasing fetal perfusate pH resulted in a doubling in meperidine transfer using an *in vivo* rabbit model.¹⁰ The three fold increase in sufentanil transfer in human placenta may suggest a potentially clinically important effect. Maternal sufentanil administration should be used with caution, particularly in premature labour, if fetal acidosis is documented or suspected. Premature infants are not only affected by the pharmacokinetics of placental transfer, but they have increased sensitivity to opioids.

Protein binding

Sufentanil transfer was decreased in the presence of fresh human plasma. Sufentanil is bound predominantly to α_1 -acid glycoprotein (AAG), and to a much lesser extent, albumin.²³ Neonatal AAG levels are considerably lower than in adults, which explains the

decreased protein binding of sufentanil in neonates.²⁴ In our study, albumin alone did not affect MTF transfer of sufentanil. Thus, sufentanil binding to AAG is the dominant force in decreasing MTF transfer, as suggested by others.²⁴ α_1 -acid glycoprotein concentration may change in response to illness. Diseases with changes in albumin concentrations (e.g. preeclampsia) should not alter sufentanil transfer.

In summary, sufentanil crossed the placenta by passive diffusion and accumulated in placental tissue, which acted as a drug depot, slowing the initial transfer. Transfer occurred within five minutes and plateaued by 60 min in both maternal-to-fetal and fetal-to-maternal directions. Placental transfer was decreased by maternal plasma proteins, presumably α_1 -acid glycoprotein, but not albumin. Fetal acidosis increased placental transfer. These characteristics of placental transfer and uptake should be considered when administering this drug during parturition, particularly during altered states of α_1 -acid glycoprotein and during suspected fetal acidosis. Due to its low initial umbilical vein concentration, sufentanil may be the opioid of choice when delivery is imminent (<45 min).

Acknowledgment

The authors would like to acknowledge the wisdom and support of Herman Turndorf MD.

References

- 1 Steinberg RB, Powell G, Hu X, Dunn SM. Epidural sufentanil for analgesia for labor and delivery. *Reg Anesth* 1989; 14: 225-8.
- 2 Camann WR, Denney RA, Holby ED, Datta S. A comparison of intrathecal, epidural, and intravenous sufentanil for labor analgesia. *Anesthesiology* 1992; 77: 884-7.
- 3 D'Angelo R, Anderson MT, Philip J, Eisenach JC. Intrathecal sufentanil compared to epidural bupivacaine for labor analgesia. *Anesthesiology* 1994; 80: 1209-15.
- 4 Cohen S, Amar D, Pantuck CB, Pantuck EJ, Goodman EJ, Leung DH. Epidural analgesia for labour and delivery: fentanyl or sufentanil? *Can J Anaesth* 1996; 43: 341-6.
- 5 Ross BK. Epidural and spinal anesthesia/analgesia. Section II: Opioid techniques. In: Chestnut DH (Ed.). *Obstetric Anesthesia Principles and Practice*. St. Louis: Mosby, 1994: 379-402.
- 6 Van Steenberge A, Debroux HC, Noorduin H. Extradural bupivacaine with sufentanil for vaginal delivery. A double-blind trial. *Br J Anaesth* 1987; 59: 1518-22.
- 7 Capogna G, Celleno D, Tomassetti M. Maternal analgesia and neonatal effects of epidural sufentanil for cesarean section. *Reg Anesth* 1989; 14: 282-7.
- 8 Loftus JR, Hill H, Cohen SE. Placental transfer and neonatal effects of epidural sufentanil and fentanyl administered

- with bupivacaine during labor. *Anesthesiology* 1995; 83: 300–8.
- 9 Vertommen JD, Marcus MAE, Van Aken H. The effects of intravenous and epidural sufentanil in the chronic maternal-fetal sheep preparation. *Anesth Analg* 1995; 80: 71–5.
- 10 Gaylard DG, Carson RJ, Reynolds F. Effect of umbilical perfusate pH and controlled maternal hypotension on placental drug transfer in the rabbit. *Anesth Analg* 1990; 71: 42–8.
- 11 Reynolds F. Principles of placental drug transfer: its measurement and interpretation. In: Reynolds F (Ed.). *The Effects on the Baby of Maternal Analgesia and Anaesthesia*. London: WB Saunders Co, 1993: 1–28.
- 12 Krishna RB, Levitz M, Dancis J. Transfer of cocaine by the perfused human placenta: the effect of binding to serum proteins. *Am J Obstet Gynecol* 1993; 169: 1418–23.
- 13 Zakowski MI, Ham AA, Grant GJ. Transfer and uptake of alfentanil in the human placenta during *in vitro* perfusion. *Anesth Analg* 1994; 79: 1089–93.
- 14 Woestenborghs RJH, Timmerman PMMBL, Cornelissen M-LJE, *et al.* Assay methods for sufentanil in plasma. Radioimmunoassay *versus* gas chromatography-mass spectrometry. *Anesthesiology* 1994; 80: 666–70.
- 15 Bansal R, Aranda JV. Simultaneous microassay of alfentanil, fentanyl, and sufentanil by high performance liquid chromatography. *J Liquid Chromatog* 1995; 18: 339–48.
- 16 Brodie BB, Axelrod J, Soberman R, Levy BB. The estimation of antipyrine in biological materials. *J Biol Chem* 1949; 179: 25–29.
- 17 Heinegard D, Tiderström G. Determination of serum creatinine by a direct colorimetric method. *Clin Chim Acta* 1973; 43: 305–10.
- 18 Epstein BS, Wurm SB. Opioids for monitored anesthesia care. In: Estafanous FG (Ed.). *Opioids in Anesthesia II*. Boston: Butterworth-Heinemann, 1991: 179–207.
- 19 Zakowski MI, Schlesinger J, Dumbroff S, Grant G, Turndorf H. In vitro human placental uptake and transfer of fentanyl. *Anesthesiology* 1993; 79: A1006.
- 20 Palot M, Visseaux H, Botmans C, Levron JC, Lemoing JP. Placental transfer and neonatal distribution of fentanyl, alfentanil, and sufentanil after continuous epidural administration for labor. *Anesthesiology* 1992; 77: A991.
- 21 Santos AC, Pedersen H, Finster M. Local anesthetics. In: Chestnut DH (Ed.). *Obstetric Anesthesia Principles and Practice*. St. Louis: Mosby, 1994: 202–28.
- 22 Johnson RF, Herman NL, Johnson HV, Arney TL, Paschall RL, Downing JW. Effects of fetal pH on local anesthetic transfer across the human placenta. *Anesthesiology* 1996; 85: 608–15.
- 23 Meuldermans WEG, Hurkmans RMA, Heykants JJP. Plasma protein binding and distribution of fentanyl, sufentanil, alfentanil and lofentanil in blood. *Arch Int Pharmacodyn* 1982; 257: 4–19.
- 24 Meistelman C, Benhamou D, Barre J, *et al.* Effects of age on plasma protein binding of sufentanil. *Anesthesiology* 1990; 72: 470–3.