

# Disposition of Bupivacaine and Its Metabolites in the Maternal, Placental, and Fetal Compartments in Rats

Hisayo O. Morishima, M.D., Ph.D.,\* Atsuro Ishizaki, M.D., Ph.D.,† Yi Zhang, M.D.,‡ Robert A. Whittington, M.D.,§ Raymond F. Suckow, Ph.D.,|| Thomas B. Cooper, M.A.#

**Background:** This study was designed to determine the disposition of bupivacaine and its metabolites in the maternal, placental, and fetal compartments, using multiple sampling time points in chronically prepared awake pregnant rats.

**Methods:** All animals received an intravenous infusion of bupivacaine at a rate of  $0.33 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  over a period of 15 min. The fetuses were delivered either at the end of infusion or at 2 or 4 h after dosing. Maternal and fetal blood and tissue samples were obtained for the assays of bupivacaine and its metabolites using capillary gas chromatography-mass spectrometry.

**Results:** The elimination half-life of bupivacaine was 37.7 min. The major metabolite was 3'-hydroxybupivacaine. Bupivacaine and 3'-hydroxybupivacaine were present in all samples at the end of administration. The fetal to maternal concentration ratio of bupivacaine in plasma was 0.29, and in the placenta was 0.63. The amnion contained the highest bupivacaine concentration: threefold higher in the maternal and 11-fold higher than in the fetal plasma. At 4 h after dosing, bupivacaine was no longer detectable in any maternal and fetal samples, whereas 3'-hydroxybupivacaine was still present in all tissues except the fetal plasma and heart.

**Conclusions:** These data indicate that a considerable amount of bupivacaine is taken up by both sides of the placenta, as well as the amnion and myometrium. 3'-Hydroxybupivacaine was present in all tissues except the fetal plasma and heart samples, even after the parent compound became no longer detectable. Whether this slow elimination of 3'-hydroxybupivacaine causes any adverse effects on the fetus-newborn needs to be explored. (Key words: Distribution; local anesthetic; mother-fetus; rodent.)

It has been a common finding that the placenta does not limit the fetal transfer of maternally administered amide-linked local anesthetics, such as lidocaine,<sup>1-3</sup> mepivacaine,<sup>4</sup> etidocaine,<sup>5</sup> bupivacaine, and ropivacaine<sup>6-8</sup> in chronically instrumented ovine model. Using an isolated, perfused human placental model, it has been suggested that bupivacaine accumulates in the placenta.<sup>9</sup> It is unknown whether this finding also applies to the metabo-

lites of bupivacaine and whether these metabolites are pharmacologically active.

The aim of the present study was to test the hypotheses that the placenta may play a significant role in the uptake of bupivacaine, that the paraplacental sites may contribute to the fetal transfer of bupivacaine, and that the metabolites of bupivacaine remain in the fetal tissue longer than in the parent compound.

## Materials and Methods

### Animals

The protocol was approved by the Columbia University Animal Care and Use Committee. Thirty pregnant Sprague-Dawley rats of known gestation were purchased from a commercial breeder (Camm, Ridgefield, NJ) 6-8 days before surgery. They were housed in a temperature-controlled room and fed Purina laboratory rodent chow (W. Fishey Feed Co., Bound Brook, NJ) *ad libitum* with water. Animals were divided into two study groups: the pharmacokinetic group (n = 8) and the transfer groups (n = 22). The latter animals were further divided into three subgroups: 0 h (n = 7), 2 h (n = 7), and 4 h (n = 8).

### Surgical Procedure

All animals underwent the same surgical procedure; the details of which have been described elsewhere.<sup>1</sup> Briefly, during general anesthesia, polyethylene catheters (PE 50, Becton Dickinson, Sparks, MD) were placed into the right carotid artery and jugular vein, 1-2 days before the study.

### Experimental Procedure

On the day of the study, the awake, near-term rat (20-22 days of gestation) was weighed and placed in a semidark, closed cardboard box. The animal was allowed to acclimate to the new environment for at least 1 h before the study (baseline). During that time, the arterial catheter was connected to a pressure transducer, and thereafter arterial blood pressure and heart rate were recorded continuously on a polygraph (Gould Electronics, Rutherford, NJ) connected to a computer. Heart rate was monitored with a cardiometer triggered by the arterial pressure.

For the pharmacokinetic study, the rat received an intravenous bolus dose of 1 mg/kg bupivacaine hydrochloride (AstraZeneca, Södertälje, Sweden), followed by an infusion rate of  $0.33 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  over a total

\* Professor, † Research Fellow, ‡ Research Associate, § Assistant Professor, Department of Anesthesiology, || Associate Research Scientist in Psychiatry, # Professor, Department of Psychiatry.

Received from the Departments of Anesthesiology and Psychiatry, College of Physicians and Surgeons, Columbia University, New York, New York. Submitted for publication December 15, 1999. Accepted for publication May 24, 2000. Supported in part by grant No. R01 DA06648 from the National Institute on Drug Abuse, Bethesda, Maryland, and grant No. MHCRC 30906 from the National Institute of Mental Health, Bethesda, Maryland. The bupivacaine metabolites were supplied by AstraZeneca, Södertälje, Sweden. Presented in part at the annual meeting of the American Society of Anesthesiologists, Dallas, Texas, October 9-13, 1999.

Address reprint requests to Dr. Morishima: Department of Anesthesiology, College of Physicians and Surgeons, Columbia University, 622 West 168th Street, PH Box # 9, New York, New York 10032. Address electronic mail to: hom2@columbia.edu. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

period of 15 min. Maternal arterial blood samples (0.3 ml) were obtained at 0, 5, 10, 15, 30, 60, 120, 180, and 240 min from the completion of the infusion. Blood samples were centrifuged to obtain plasma and treated in the same manner as in the transfer study described below.

For the transfer study, all animals received an infusion of bupivacaine at a rate of  $0.33 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  over a period of 15 min. This dosing was calculated to obtain a maximum concentration of bupivacaine in the maternal plasma of 1,200–1,400 ng/ml, which is similar to plasma concentrations of bupivacaine in pregnant women receiving epidural bupivacaine for cesarean section.<sup>11</sup> Maternal arterial blood was withdrawn either at one of three time points: at the completion of the drug infusion (0 h) or at 2 or 4 h after infusion. At each sampling point, the animal was placed in a beaker containing gauze sponges that were saturated with isoflurane. As soon as the animal became unconscious (within 30 s), the maternal heart was removed, the uterine horns were exteriorized, and all of the blood vessels supplying the uterus were clamped. The maternal brain and liver were removed, and proper catheter placement was verified. Amniotic fluid, corresponding to the harvesting fetuses, was aspirated. The number of fetuses in each horn was counted, and fetal blood samples were obtained *via* cardiac puncture. The following tissues were then obtained and immediately frozen on dry ice: the uterus, amnion, the maternal and fetal side of the placenta, as well as the fetal brain, heart, and liver. On the basis of our preliminary experiments, drug concentrations obtained from the fetuses located in the middle, distal, and proximal uterine cavities were similar, and the samples obtained from both sides of the uterine horns were also similar. Therefore, several fetal samples harvested from the same horn were pooled, making the size of each fetal sample twice as large as the corresponding maternal tissue size. Blood was centrifuged to obtain plasma, and all samples were frozen on dry ice and stored at  $-70^\circ\text{C}$  for subsequent measurement of concentrations of bupivacaine and its metabolites.

#### *Analysis of Bupivacaine and Its Metabolites*

Plasma and tissue concentrations of bupivacaine and its metabolites were analyzed *via* liquid chromatography with spectrophotometric (ultraviolet) detection as described by Kastrissions *et al.*<sup>12</sup> Plasma or tissue homogenate to which has been added the internal standard (bupropion) are made alkaline and extracted with *t*-methyl-butyl ether, and the organic phase is then back extracted into 0.1 M hydrochloride. The aqueous acid phase is dried *via* vacuum centrifuge, the residue is reconstituted in the mobile phase, and an aliquot is injected. The eluent was monitored at a wavelength of 210 nm. Calibration curves for bupivacaine and each of the three metabolites were linear across the entire concentration range of the samples. A detection limit of 5 ng/ml for

all compounds was obtained. Relative SD% for the high (500 ng/ml), medium (150 ng/ml), and low (25 ng/ml) quality-control concentrations were 4.7, 3.4, and 9.5 for bupivacaine; 4.1, 3.1, and 6.0 for desbutylbupivacaine; 3.8, 3.1, and 4.0 for 3'-hydroxybupivacaine; and 3.9, 3.5, and 4.8 for 4'-hydroxybupivacaine, respectively.

#### *Free Drug Concentrations*

Assays were conducted within 1 week of sample collection. Serum pH was first adjusted to  $7.45 \pm 0.02$  pH units, which is within the physiological range for pregnant rats. Different amounts of bupivacaine were added to three aliquots of this plasma to obtain concentrations of up to 2,000 ng/ml for bupivacaine. The ultrafiltration assays were conducted in triplicate using Amicon MPS-5 with YMT membranes (Amicon Corp., Danvers, MA) and drug concentration in serum and serum water was determined as described above. Equilibrium dialysis of serum and the ultrafiltration technique produced comparable results. Nonspecific binding of local anesthetics to these membranes did not occur.<sup>6,7</sup> YMT membranes were capable of removing 99.9% of serum protein while maintaining serum pH within 0.1 units.

#### *Pharmacokinetic Data Analysis*

Maternal blood samples were collected at predetermined time intervals from 0 to 240 min after infusion. Because of the small blood volume, fetal samples were obtained only at 0, 2, or 4 h after infusion. The maternal values were used as input data for the pharmacokinetic computer program WinNonlin, Version 2.1 (Pharsight Corporation, Palo Alto, CA) to provide a nonlinear least squares regression fit for these data. The pharmacokinetic analysis of bupivacaine assumed a two-compartment open model as previously reported.<sup>6,7</sup>

#### *Statistical Analysis*

Data were compared using repeated-measures analysis of variance for between group difference and changes in heart rate and arterial pressure over time. Comparison between maternal and fetal drug concentrations were performed using the Student paired *t* test, whereas comparisons between three-time groups were performed using unpaired *t* tests. All results are expressed as mean  $\pm$  SD, and a *P* value less than 0.05 was considered statistically significant.

## **Results**

The mean gestational age ( $\pm$  SD) was  $21 \pm 1$  days, and the maternal and fetal weights were comparable in all groups, with mean values of  $320 \pm 28$  g and  $3.1 \pm 0.4$  g, respectively.

#### *Physiologic Responses*

Baseline heart rate and mean arterial pressure in all mothers were similar:  $392 \pm 44$  beats/min and  $137 \pm 11$  mmHg,

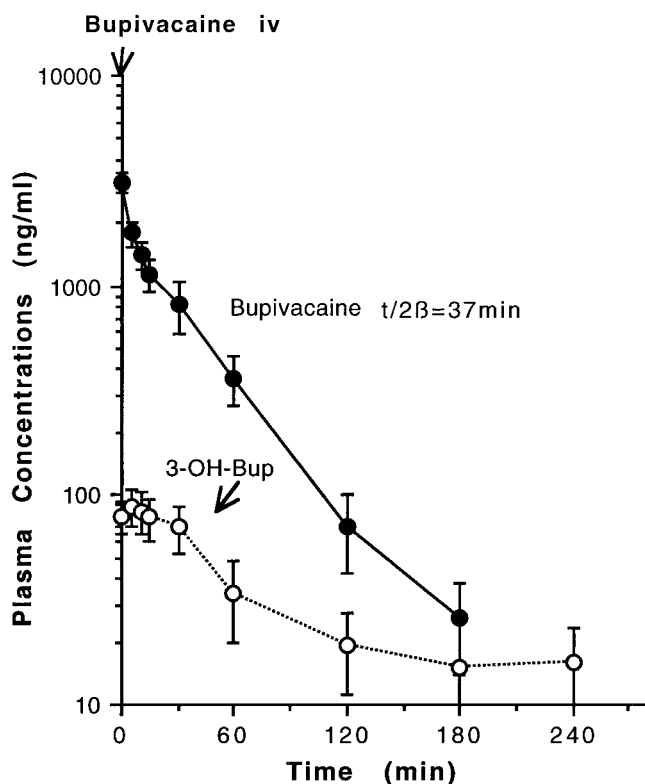


Fig. 1. Plasma concentrations of bupivacaine and 3'-hydroxybupivacaine (3'-OH-Bup) after an intravenous bolus dose of 1 mg/kg bupivacaine hydrochloride, followed by infusion at a rate of  $0.33 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  over a total period of 15 min to pregnant rats.

respectively. A transient decrease in the heart rate occurred during bupivacaine infusion with a maximum change to  $342 \pm 42$  beats/min in the kinetic study, and  $355 \pm 39$  beats/min in the transfer study. However, none of these values was statistically different from baseline. Behavioral alterations caused by bupivacaine infusion were not noticeable; all animals remained quiet throughout.

#### Bupivacaine and Its Metabolites

**Pharmacokinetic Data.** The mean peak bupivacaine concentration in the maternal plasma,  $3,123 \pm 370$  ng/ml, was measured in the sample drawn at the end of infusion. Subsequently, the concentration decreased steadily to  $26 \pm 12$  ng/ml at 180 min and became undetectable by 240 min (fig. 1). 3'-Hydroxybupivacaine was the major metabolite among the three metabolites, appearing in the first plasma sample obtained at the end of infusion at a lower concentration,  $80 \pm 14$  ng/ml, than the corresponding bupivacaine value. However, it decreased more slowly over time; the mean concentrations at 180 and 240 min were  $16 \pm 11$  and  $15 \pm 7$  ng/ml, respectively. In plasma, 4'-hydroxybupivacaine and 2,6-pipecoloxylidide were not detected.

The pharmacokinetic profile of bupivacaine was as follows: distribution half-life,  $1.2 \pm 0.1$  min; elimination half-life,  $37.7 \pm 2.4$  min; volume of distribution at

steady state,  $3.86 \pm 0.29$  l/kg; and total clearance,  $93.3 \pm 8.6 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ; K12,  $0.462 \pm 0.066$ ; K21,  $0.081 \pm 0.008$ , and K10,  $0.166 \pm 0.021$ .

**Transfer Data.** Mean concentrations for bupivacaine and its metabolites in maternal and fetal plasma, tissues, maternal and fetal sides of the placenta, as well as paraplacental tissues obtained at three different postinfusion times are summarized in table 1. Because, in most samples, 4'-hydroxybupivacaine and 2,6-pipecoloxylidide were present either only in trace amounts or no longer detected by 2 h after infusion, their 2- and 4-h data were excluded from the table. The peak maternal plasma bupivacaine concentration,  $1,217 \pm 130$  ng/ml, was measured in the sample drawn at the end of infusion. This concentration decreased significantly to  $115 \pm 32$  ng/ml ( $P < 0.001$ ) by the 2-h postinfusion period, becoming virtually undetectable by 4 h after infusion. A relatively high concentration of bupivacaine was measured in the maternal brain,  $3,350 \pm 686$  ng/g at 0 h, but it decreased rapidly and became unmeasurable by 4 h. In the fetal plasma, a peak bupivacaine concentration of  $320 \pm 38$  ng/ml also decreased rapidly; only a trace amount was detected at 2 h and the drug became undetectable by 4 h after infusion. A bupivacaine concentration of  $4,817 \pm 976$  ng/g in the amnion at the end of infusion was the highest among all samples obtained at any time.

Similar to the maternal and fetal plasma or tissues, bupivacaine in the placenta, amnion, and myometrium concentrations decreased rapidly. Bupivacaine concentrations in maternal plasma and tissue samples were significantly higher than in the corresponding fetal samples. The fetal to maternal plasma concentration ratio of bupivacaine at 0 h was  $0.29 \pm 0.04$ , whereas the fetal to maternal placental concentration ratio was  $0.63 \pm 0.06$ . Bupivacaine concentration,  $226 \pm 32$  mg/ml, in the amniotic fluid at the end of infusion was similar to the fetal plasma concentration. 3'-Hydroxybupivacaine was present in all samples in lower concentrations than bupivacaine at 0 h with the exception of the maternal liver in which concentrations of both compounds were similar. 4'-Hydroxybupivacaine was also present in all bupivacaine samples in the fetal plasma and heart. 2,6-Pipecoloxylidide was a minor metabolite (table 2).

Generally, the 3'-hydroxybupivacaine concentration was lower than the corresponding parent compound obtained at 0 h. However, in contrast to the rapidly decreasing bupivacaine concentrations, 3'-hydroxybupivacaine in all but the fetal plasma and heart tissue remained at significantly high concentrations, which resulted in an increased concentration ratio between this metabolite and the parent compound. The 3'-hydroxybupivacaine concentration eventually exceeded the parent compound. Sustained concentrations of this metabolite were found even after bupivacaine was no longer detectable by 4 h. The concentration of 3'-hydroxybupivacaine in both the maternal and fetal liver at 0 h ex-

**Table 1. Mean Values ( $\pm$  SD) for Concentrations of Bupivacaine and Metabolites (ng/ml or ng/g) in Pregnant Rats and Their Fetuses at 0-h, 2-h or 4-h Postmaternal Infusion of Bupivacaine ( $0.33 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) over 15 min**

Sample	End of Infusion (N = 7)				2-h Postinfusion (N = 7)		4-h Postinfusion (N = 8)	
	Bupivacaine	3-OH Bup	4-OH Bup	PPX	Bupivacaine	3-OH Bup	Bupivacaine	3-OH Bup
<b>Mother</b>								
Plasma	1,217 $\pm$ 130	39 $\pm$ 4	T	ND	115 $\pm$ 32	24 $\pm$ 10	ND	54 $\pm$ 26
Brain	3,350 $\pm$ 686	111 $\pm$ 21	93 $\pm$ 63	ND	203 $\pm$ 77	87 $\pm$ 64	ND	71 $\pm$ 68
Heart	1,906 $\pm$ 372	109 $\pm$ 46	T	ND	289 $\pm$ 72	290 $\pm$ 70	ND	273 $\pm$ 64
Liver	2,684 $\pm$ 718	2,423 $\pm$ 322	184 $\pm$ 27	107 $\pm$ 55	147 $\pm$ 77	1,357 $\pm$ 138	ND	1,236 $\pm$ 564
<b>Fetus</b>								
Plasma	320 $\pm$ 38	21 $\pm$ 6	ND	ND	T	T	ND	ND
Brain	1,070 $\pm$ 227	70 $\pm$ 15	47 $\pm$ 7	ND	91 $\pm$ 60	38 $\pm$ 16	ND	31 $\pm$ 15
Heart	789 $\pm$ 114	T	ND	ND	T	T	ND	ND
Liver	1,041 $\pm$ 187	141 $\pm$ 28	T	T	38 $\pm$ 20	209 $\pm$ 132	ND	46 $\pm$ 18
<b>Placenta</b>								
Maternal	3,440 $\pm$ 413	191 $\pm$ 28	173 $\pm$ 73	ND	393 $\pm$ 126	227 $\pm$ 47	88 $\pm$ 55	223 $\pm$ 183
Fetal	2,163 $\pm$ 339	166 $\pm$ 42	90 $\pm$ 57	T	160 $\pm$ 85	162 $\pm$ 33	ND	166 $\pm$ 106
Amnion	4,817 $\pm$ 976	390 $\pm$ 114	593 $\pm$ 102	182 $\pm$ 70	760 $\pm$ 86	371 $\pm$ 177	303 $\pm$ 76	449 $\pm$ 63
Amniotic fluid	226 $\pm$ 32	T	T	ND	77 $\pm$ 57	37 $\pm$ 31	ND	23 $\pm$ 6
Myometrium	1,906 $\pm$ 243	564 $\pm$ 138	281 $\pm$ 89	T	413 $\pm$ 170	276 $\pm$ 61	96 $\pm$ 36	302 $\pm$ 187

3-OH Bup = 3'-hydroxybupivacaine; 4-OH = 4'-hydroxybupivacaine; PPX = 2,6-pipecolylxylidide; T = trace amount; ND = not detected.

ceeded the parent compound by 2 and 4 h after dosing. Figure 2 illustrates the relation between concentrations of the parent compound and its metabolites in the maternal and fetal placenta over time after maternal administration of bupivacaine. While the bupivacaine concentration steadily decreased, the 3'-hydroxybupivacaine concentration remained virtually constant. In the amnion and myometrium, 3'-hydroxybupivacaine also kept increasing, becoming higher than the corresponding bupivacaine concentration. Despite high concentrations of 3'-hydroxybupivacaine in these tissues, the amniotic fluid concentration was not as high as expected. 4'-Hydroxybupivacaine and 2,6-pipecolylxylidide were only detected in trace amounts or not at all in samples obtained at 2 h.

## Discussion

This *in vivo* study demonstrates the distribution of maternally administered bupivacaine and its metabolites

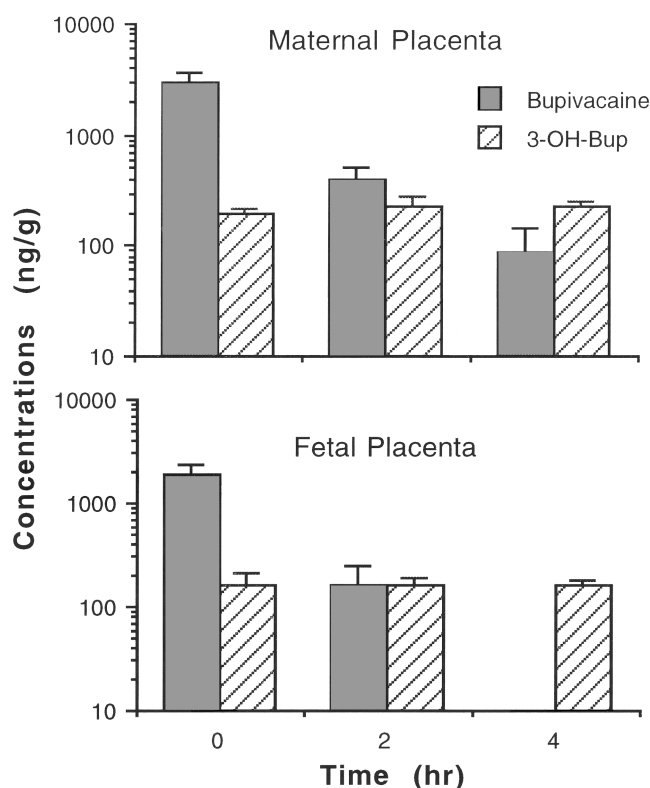
in the maternal and fetal tissues in which samples were obtained at multiple time points. The peak maternal concentration of bupivacaine was similar to that reported in the pregnant and nonpregnant women receiving bupivacaine epidurally.<sup>11</sup> In that study, a measurable concentration of 4'-hydroxybupivacaine appeared in serum 30 min after bupivacaine administration, and gradually increased, peaking between 2 and 6 h after dosing, whereas the concentration of the parent compound declined rapidly. However, nonmeasurable conjugated 4'-hydroxybupivacaine was found in the serum of pregnant subjects. A delayed detection of this metabolite also occurred in our pregnant rats, in which 4'-hydroxybupivacaine became measurable in the plasma 30–45 min after the intravenous administration of bupivacaine. We found that the major metabolite of bupivacaine in rats is 3'-hydroxybupivacaine. Caldwell *et al.*<sup>15</sup> made the same observation after intraperitoneal administration of bupivacaine to the rat.

Our pharmacokinetic profile of bupivacaine was differ-

**Table 2. Mean ( $\pm$  SD) Concentrations (ng/g) of Bupivacaine and Its Metabolites in the Maternal and Fetal Liver and Placenta at Various Sampling Times**

Postinfusion Time (h)	Liver			Placenta		
	0	2	4	0	2	4
<b>Mother</b>						
Bupivacaine	2,684 $\pm$ 718	147 $\pm$ 77	ND	3,440 $\pm$ 413	393 $\pm$ 126	88 $\pm$ 55
3-OH Bup	2,423 $\pm$ 322	1,357 $\pm$ 138	1,236 $\pm$ 564	191 $\pm$ 28	227 $\pm$ 47	223 $\pm$ 183
4-OH Bup	184 $\pm$ 27	51 $\pm$ 34	28 $\pm$ 18	173 $\pm$ 73	ND	61 $\pm$ 42
PPX-HCl	107 $\pm$ 55	ND	65 $\pm$ 34	ND	ND	32 $\pm$ 18
<b>Fetus</b>						
Bupivacaine	1,041 $\pm$ 187	38 $\pm$ 20	ND	2,163 $\pm$ 339	160 $\pm$ 85	ND
3-OH Bup	141 $\pm$ 28	209 $\pm$ 132	46 $\pm$ 18	166 $\pm$ 42	162 $\pm$ 33	166 $\pm$ 106
4-OH Bup	T	ND	ND	90 $\pm$ 57	ND	ND
PPX-HCl	T	ND	ND	T	ND	ND

ND = not detected; 3-OH Bup = 3'-hydroxybupivacaine; 4-OH Bup = 4'-hydroxybupivacaine; PPX-HCl = 2,6-pipecolylxylidide hydrochloride; T = trace amount.



**Fig. 2.** Concentrations of bupivacaine and 3'-hydroxybupivacaine (3-OH-Bup) in the maternal and fetal placentae at the completion of bupivacaine infusion (0 h), and 2 and 4 h after dosing of bupivacaine to the pregnant rats.

ent from that reported in the rat by Drenth *et al.*,<sup>14</sup> possibly partly because of a difference in the mode of drug administration. In their study, 5.5 mg/kg of bupivacaine was injected intravenously as a bolus dose, reaching a toxic blood concentration of 7,900 ng/ml after 1 min. The concentration decreased rapidly to 350 ng/ml by 10 min and became undetectable by 120 min, indicating a shorter elimination half-life of 24.7 min. In the present study, a similar dose of the drug was administered over a period of 15 min, producing a peak concentration of 1,217 ng/ml and a half-life of 37.7 min. Recent pharmacokinetic data in pregnant parturients receiving a mixture of bupivacaine and fentanyl epidurally showed the elimination half-life of bupivacaine to be 87 min,<sup>15</sup> with maximum maternal and fetal plasma concentrations of 460 ng/ml and 260 ng/ml, respectively. In pregnant ewes, the bupivacaine half-life of 102.2 min after intravenous infusion of the drug over a period of 15 min<sup>6</sup> was longer than corresponding values in human parturients and rats. These differences may be a result of differences in experimental protocols as well as the species-related differences.

In the transfer study, all maternal samples had higher bupivacaine concentrations compared with the fetus; this was particularly significant in the plasma and brain, where the fetal to maternal concentration ratios at the end of the administration were  $0.29 \pm 0.04$  and  $0.32 \pm$

$0.04$ , respectively. Our finding of the fetal to maternal concentration ratio of bupivacaine is in agreement with data obtained from pregnant women receiving bupivacaine for epidural anesthesia.<sup>15,16</sup> In contrast to these large maternal to fetal concentration gradients, differences in bupivacaine concentrations between the maternal and fetal placenta were not statistically significant. A small fetal to maternal plasma concentration gradient and a high placental uptake of bupivacaine accompanied by a large fetal to maternal placental concentration ratio of  $0.63 \pm 0.05$  suggest that bupivacaine is retained in the placenta, thus limiting its transfer between the maternal and fetal compartments.

The placental transfer of bupivacaine has been studied in several species. It has been well documented that physiochemical factors influence the transfer of bupivacaine to the fetus, including its high lipid solubility and high protein binding. A decrease in the fetal pH will also affect the uptake of a basic drug, such as bupivacaine. During general anesthesia, fetal rabbits were delivered one by one with 10–15-min intervals during maternal intravenous infusion of bupivacaine.<sup>17</sup> While bupivacaine in the maternal plasma remained at a steady concentration, the fetal as well as amniotic fluid concentrations increased progressively. Although pH was not measured in their study, repeated hysterotomies may have caused uterine contractions, thus decreasing the uteroplacental blood flow and oxygen delivery to the fetus, resulting in fetal asphyxia, which will trap bupivacaine.

Little attention has been paid to the metabolites of bupivacaine. In the present study, bupivacaine disappeared rapidly from both the maternal and fetal circulation as well as from both sides of the placenta and the amnion. There was a substantial accumulation of metabolites, mainly 3'-hydroxybupivacaine, in the maternal and fetal tissues. Although a pharmacokinetic study of the metabolites was not performed in the present study, it can be assumed from our results that the major metabolite, 3'-hydroxybupivacaine, has a significantly longer elimination half-life.

In the fetus, 3'-hydroxybupivacaine remained detectable in the liver for as long as 4 h, whereas it virtually disappeared from other tissues. The association of a high fetal hepatic concentration of 3'-hydroxybupivacaine with a declining bupivacaine concentration may be the result of several factors. The major part of umbilical venous blood perfuses the fetal liver; therefore, placentally transmitted bupivacaine may directly enter the fetal liver, where the concentration of the breakdown product of this compound is increasing while the concentration of the parent compound is decreasing. This would suggest that the fetal liver is capable of metabolizing bupivacaine. However, because polar metabolites do not readily cross the placental membrane bidirectionally, 3'-hydroxybupivacaine likely accumulates in the fetal

side. Another noticeable finding in this study was the slow elimination of 3'-hydroxybupivacaine by the placenta. A greater placental concentration ratio over the time course may have considerable effects on the developing fetus-neonate if this metabolite has pharmacologic activity.

Furthermore, a distinct finding was that the highest concentrations of bupivacaine and 3'-hydroxybupivacaine were present in the amnion. The fetal membranes (amnion) line the uterine cavity and completely surround the fetus, producing the amniotic fluid. The high affinity of bupivacaine as well as 3'-hydroxybupivacaine for this membrane indicates that the amnion plays a significant role in retaining these compounds and may continue to deliver them to the amniotic fluid *via* diffusion, even after they have become undetectable in the maternal circulation. However, concentrations of bupivacaine and its metabolites in the amniotic fluid were much lower as compared with the values obtained in the amnion. These differences are likely to be caused by the large volume of amniotic fluid, diluting high concentrations of compounds. It should be noted that if bupivacaine is continuously or repeatedly administered, this drug, particularly 3'-hydroxybupivacaine, could accumulate in the amniotic fluid. This finding was first observed with cocaethylene when it was being administered to the pregnant rat.<sup>10</sup> High concentrations of bupivacaine and the prolonged retention of 3'-hydroxybupivacaine were also found in the myometrium, suggesting that the paraplacental site also plays an important role in accumulating these compounds.

Although it has not been established whether 3'-hydroxybupivacaine is detected or has any pharmacologic activity in the human, the present findings indicate that a long-lasting 3'-hydroxybupivacaine in the rat may hypothetically accumulate during prolonged administration.

In conclusion, a considerable amount of bupivacaine and 3'-hydroxybupivacaine are taken up by the maternal and fetal placentae and retained in these tissues. The amnion and myometrium also have a high affinity for bupivacaine, which may contribute to the nonplacental supply of this drug to the fetus.

The authors thank Ralph M. Richart, M.D., Professor of Pathology and Obstetrics/Gynecology at Columbia University, for the histologic identification of the maternal and fetal sides of the placenta and the amnion and myometrium of rats, and K. Khan, B.S., and M. Fein, B.S., for technical assistance.

## References

1. Morishima HO, Heymann MA, Rudolph AM, Barrett CT, James LS: Transfer of lidocaine across the sheep placenta to the fetus: Hemodynamic and acid-base responses of the fetal lamb. *Am J Obstet Gynecol* 1975; 122:581-8
2. Morishima HO, Finster N, Pedersen H, Fukunaga A, Ronfeld RA, Vassallo HG, Covino BG: Pharmacokinetics of lidocaine in fetal and neonatal lambs and adult sheep. *ANESTHESIOLOGY* 1979; 50:431-6
3. Santos AC, Pedersen H, Morishima HO, Finster M, Arthur GR, Covino BG: Pharmacokinetics of lidocaine in nonpregnant and pregnant ewes. *Anesth Analg* 1988; 67:1154-8
4. Morishima HO, Adamsons K Jr: Placental clearance of mepivacaine following its administration to the fetus. *ANESTHESIOLOGY* 1967; 28:343-8
5. Pedersen H, Morishima HO, Finster M, Arthur GR, Covino BG: Pharmacokinetics of etidocaine in fetal and neonatal lambs and adult sheep. *Anesth Analg* 1982; 61:104-8
6. Santos AC, Arthur GR, Lehning E, Finster M: Comparative pharmacokinetics of ropivacaine and bupivacaine in nonpregnant and pregnant ewes. *Anesth Analg* 1997; 84:873-93
7. Santos AC, Pedersen H, Sallusto JA, Johnson HV, Morishima HO, Finster M, Arthur GR, Covino BG: Pharmacokinetics of ropivacaine in nonpregnant and pregnant ewes. *Anesth Analg* 1990; 7:262-6
8. Kennedy RL, Bell JU, Miller RP, Doshi D, deSousa H, Kennedy MJ, Heald D, David J: Uptake and distribution of bupivacaine in fetal lambs. *ANESTHESIOLOGY* 1986; 65:247-53
9. Johnson RF, Herman N, Arney T, Gonzalez H, Johnson HV, Downing JW: Bupivacaine transfer across the human term placenta: A study using the dual perfused human placental model. *ANESTHESIOLOGY* 1995; 85:459-68
10. Morishima HO, Whittington RA, Zhang Y, Cooper TB: The disposition of cocaethylene in rat maternal, placental, and fetal compartments. *Am J Obstet Gynecol* 1999; 180:1289-96
11. Pihlajamaki K, Kanto J, Lindberg R, Karanko M, Kiilholma P: Extradural administration of bupivacaine: Pharmacokinetics and metabolism in pregnant and non-pregnant women. *Brit J Anaesth* 1990; 64:556-62
12. Kastrissians H, Hung M-F, Triggs EJ: High-performance liquid chromatographic method for the quantitation of bupivacaine, 2,6-pipecoloxylidide and 4/E-hydroxybupivacaine in plasma and urine. *J Chromatogr* 1992; 577:103-7
13. Caldwell J, Notarianni LJ, Smith RL, Snedden W: The metabolism of bupivacaine in the rat. *Brit J Pharmacol* 1977; 61:135-6
14. Dennhardt R, Fincke M, Stockert G: Metabolism and distribution of bupivacaine-experiments in rats II. Distribution and elimination. *Reg Anaesth* 1978; 1:86-92
15. Decocq G, Brazier M, Hary M, Hubau C, Fortaine MR, Gondry J, Andreja M: Serum bupivacaine concentrations and transplacental transfer following repeated epidural administrations in term parturients during labour. *Fundam Clin Pharmacol* 1997; 11:365-70
16. 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
17. Laisley RS, Carson RJ, Reynolds F: Effect of adrenaline on the distribution of bupivacaine in the rabbit fetus. *Br J Anaesth* 1989; 63:721-5