

Does Pregnancy Alter the Systemic Toxicity of Local Anesthetics?

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The toxicity of mepivacaine in chronically instrumented non-pregnant and pregnant sheep was evaluated, and compared with data from previous studies of the toxicity of other local anesthetics. Thirteen preparations were studied, seven nonpregnant (NP) and six pregnant (P). Mepivacaine $2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ was infused at a constant rate into the femoral vein until toxic manifestations occurred, in the following sequence: convulsions, hypotension, respiratory arrest, and circulatory collapse. The doses and plasma concentrations of mepivacaine necessary to produce toxic symptoms were similar in NP and P animals, whereas, in a previous study, pregnancy enhanced the cardiotoxicity of bupivacaine. No malignant ventricular arrhythmias were observed throughout the study. Protein binding of mepivacaine was also determined in sera from nonpregnant and pregnant ewes and compared with that for bupivacaine. Serum protein binding of mepivacaine was not reduced in pregnancy at the drug concentrations associated with toxic symptoms; at circulatory collapse, it was approximately 22% in NP and P. In contrast, the proportion of bound bupivacaine was 73% in NP and 51% in P, a significant difference. These protein binding data suggest that, although lethal concentrations of bupivacaine, determined in the previous study, were higher in NP than in P animals, concentrations of free drug were similar. Thus, the difference between the two drugs may be related to gestational increases in the availability of free drug in the case of bupivacaine. (Key words: Anesthesia: obstetrics. Anesthetics, local: mepivacaine; toxicity. Protein binding: mepivacaine.)

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CONSIDERABLE EVIDENCE has accumulated indicating that the long-acting amide local anesthetics, such as bupivacaine, may be more cardiotoxic than agents with an intermediate duration of action, such as lidocaine. Studies conducted in various conscious animal preparations (mice, dogs, and sheep), have shown that the ratio of the dose required to produce cardiovascular collapse *versus* that resulting in convulsions (CC/CNS ratio) is lower for bupivacaine compared with that for lidocaine.¹⁻³ In humans, accidental intravenous injection of bupivacaine can result in cardiovascular collapse, which may be refractory to resuscitation.⁴ Most of these reported cases have occurred in parturients during attempted induction of epidural anesthesia for cesarean section or labor.

The question arose whether the relatively high incidence of cardiovascular collapse following bupivacaine intoxication in pregnant patients is due to the widespread use of this agent in obstetrics, or whether pregnancy enhances vulnerability to the cardiotoxicity of local anesthetics. It has been demonstrated that pregnant sheep receiving a continuous intravenous infusion of bupivacaine are, in fact, more sensitive to the toxic effects of the drug, in that the mean dose of bupivacaine required to produce cardiovascular collapse, and the plasma concentrations of bupivacaine associated with it, were lower in the pregnant ewe compared with those in the nonpregnant animal.³

The current study was undertaken to ascertain whether the greater sensitivity of the pregnant animal to local anesthetics is unique to bupivacaine, or applies to other amide agents as well. Mepivacaine was chosen because it is a homologue of bupivacaine, not uncommonly used for regional anesthesia during pregnancy and parturition.^{5,6}

Protein binding of mepivacaine and bupivacaine was also determined in sera obtained from nonpregnant and pregnant ewes in order to evaluate the contribution of altered protein binding in pregnancy to local anesthetic toxicity.

Materials and Methods

Seven nonpregnant and six pregnant sheep, near term, were used, under a protocol approved by the Institutional Animal Care and Use Committee. The details of surgical preparation have been described previously.⁷ Briefly, ewes were given spinal anesthesia, using tetracaine hydrochloride, 10-12 mg. A femoral cutdown was then performed

TABLE 1. Mean (\pm SE) Time to Onset of Toxic Manifestations (Min)

	Nonpregnant	Pregnant	P
Convulsions (CNS)	17.9 \pm 1.7	19.2 \pm 3.4	NS
Hypotension	26.9 \pm 3.3	34.4 \pm 4.6	NS
Apnea	27.0 \pm 3.2	37.7 \pm 5.0	NS
Circulatory collapse (CC)	28.6 \pm 3.3	38.5 \pm 5.1	NS

and polyethylene catheters inserted into the femoral artery and vein. The animals were allowed to recover for at least 3 days.

On the day of the study, ewes were placed in a cart, free to stand or lie down, and electrocardiogram electrodes were attached to their extremities. Heart rate and arterial blood pressure were monitored, and recorded continuously, on a polygraph. ECG and respirations were recorded periodically. Throughout the experiment, mepivacaine hydrochloride (Carbocaine[®]), was infused into the femoral vein of all animals at a constant rate of approximately 2 mg \cdot kg⁻¹ \cdot min⁻¹. Arterial blood samples were obtained at the onset of each toxic manifestation, which occurred in sequence, as previously described; convulsions, followed by hypotension, apnea, and circulatory collapse, defined as absence of blood pressure.^{3,7,8} After determination of blood pH and gas tensions, blood samples were centrifuged, and plasma separated and frozen until drug analysis.

At circulatory collapse, the brain and heart were dissected, and samples of these organs were weighed and frozen until drug analysis. Arterial plasma and tissue concentrations of mepivacaine were determined by a gas chromatographic technique similar to that previously described by Tucker.⁹ The sensitivity of this assay was 0.02 μ g/ml; the coefficient of variation ranged from 10% at 0.02 μ g/ml to 7% at 1.00 μ g/ml during the course of this study. The mean dose administered, and the plasma concentration of mepivacaine, at each toxic manifestation, as well as mean tissue concentrations of drug at cardiovascular collapse in nonpregnant and pregnant animals, were compared. Student's *t* test for unpaired data was used to determine significant differences between the pregnant and nonpregnant animals. Power analysis was applied as necessary; a meaningful difference was considered to be of the same order of magnitude as seen with bupivacaine.³ Where repeated testing was performed, a correction factor increasing the threshold for statistical significance was used. Analysis of variance (Fisher PLSD test) was used to evaluate differences from control in heart rate, blood pressure, blood pH, and gas tensions at each toxic manifestation. All results are reported as mean \pm SE.

For protein binding determinations, pooled sera from either nonpregnant or pregnant sheep at gestational age greater than 130 days (term 148 days) were obtained from the breeder, who was instructed to adhere to the following

protocol: blood obtained by venipuncture from animals unexposed to any drugs was allowed to clot for approximately 30 min, contact with polystyrene plastic or stoppers containing TBEP plasticizer being avoided. After centrifugation, the sera were frozen at -20° C until used. Serum, rather than plasma, was chosen in order to avoid the artifactual effects of *in vitro* lipolysis which is particularly significant in plasma of pregnant animals.^{10,11}

Studies were conducted within 1 week of blood collection. Serum pH was first adjusted with hydrochloric acid or sodium hydroxide to be 7.50 ± 0.02 pH units, which is physiologic for the ovine species. Local anesthetic was added to 10-ml aliquots of serum. Serum drug concentrations were chosen to be within the range of those observed during the toxicity studies involving bupivacaine and mepivacaine. The serum and drug were allowed to equilibrate for at least 1 h at room temperature. Using an ultrafiltration system, the Amicon[®] MPS-1 with YMT membranes, serum water was obtained from 1-ml aliquots of serum. Three replicate samples were tested at each concentration. Centrifugation was for 45 min at 2000 \times g. Thereafter, drug concentrations in serum and serum water were determined as previously stated. Equilibrium dialysis of serum and this ultrafiltration technique produced comparable results, and nonspecific binding of local anesthetics to the membrane did not occur.¹² YMT membranes are capable of removing 99.9% of serum protein while maintaining serum pH within 0.1 units. Unpaired Student's *t* test was used to detect differences between protein binding of drugs in sera from pregnant and nonpregnant animals. A correction factor for repeated testing was used, a *P* value of less than 0.0166 being considered significant.

Results

Pregnant sheep were studied at 128 ± 3 days of gestation. Their weight, 47.8 ± 4.3 kg, was similar to that of the nonpregnant ewes, 46.7 ± 1.9 kg. Mean heart rate, arterial blood pressure, and arterial pH and blood gas tensions were normal for our laboratory, and similar for the two groups. During mepivacaine infusion, manifestations of local anesthetic toxicity occurred in all animals, in the same sequence, as previously described for lidocaine, etidocaine, and bupivacaine.^{3,7,8} Toxic manifestations occurred after similar intervals in both groups of animals (table 1). Convulsions were the first sign, followed by hypotension, respiratory arrest, and, finally, cardiovascular collapse. Seizure activity occurred after similar drug doses in both the nonpregnant and pregnant group, 7.5 ± 1.0 mg/kg and 7.8 ± 0.7 mg/kg (table 2). At the onset of seizures, mean heart rate increased significantly over control values from 115 ± 11 to 209 ± 17 beats per minute (bpm) in the nonpregnant, and from 94 ± 9 to

TABLE 2. Mean (\pm SE) Dosages (mg/kg) and Plasma Concentrations (μ g/ml) of Mepivacaine in Nonpregnant and Pregnant Ewes at the Onset of Each Toxic Manifestation

	Nonpregnant		Pregnant	
	Dosage	Concentration	Dosage	Concentration
Convulsions (CNS)	7.5 \pm 1.0	17.4 \pm 1.7	7.8 \pm 0.7	19.5 \pm 0.7
Hypotension	48.9 \pm 5.8	36.4 \pm 2.5	62.8 \pm 7.7	38.2 \pm 3.3
Apnea	50.8 \pm 5.8	38.2 \pm 2.2	67.9 \pm 8.2	42.3 \pm 6.2
Circulatory collapse (CC)	51.7 \pm 5.3	41.6 \pm 2.4	69.4 \pm 8.3	45.6 \pm 6.2
CC/CNS ratio	7.6 \pm 0.4	2.5 \pm 0.2	9.2 \pm 1.3	2.4 \pm 0.3

Differences between groups not significant.

193 \pm 15 bpm in the pregnant ewe (table 3). Mean arterial blood pressure likewise increased from 93 \pm 2 to 175 \pm 8 mmHg in the nonpregnant, and from 92 \pm 6 to 151 \pm 7 mmHg in the pregnant sheep.

Animals in both groups developed tachypnea with the onset of convulsions, respiratory rate increasing by 40–50%. This rate decreased rapidly shortly before the onset of hypotension, at which time it was approximately 30% below the control values.

In the nonpregnant ewe, arterial pH, P_{CO₂}, and P_{O₂}, were unchanged until hypotension ensued (table 4). In the pregnant animal, the P_{O₂} was already significantly lower at the onset of convulsions, while the pH and P_{CO₂} did not change significantly until hypotension occurred.

As infusion continued, hypotension occurred after a similar drug dose in both groups; 48.9 \pm 5.8 mg/kg in the nonpregnant and 62.8 \pm 7.7 mg/kg in the pregnant

animal (table 2). Circulatory collapse quickly followed apnea in all animals. This occurred at a mean dose of 51.7 \pm 5.3 mg/kg in the nonpregnant ewe, and was similar to that in pregnant animals, 69.4 \pm 8.3 mg/kg. The ratio of the dosages resulting in cardiovascular collapse and those required for convulsive activity (CC/CNS) were also not different. The power of the test for detecting a meaningful difference in the dose required to produce circulatory collapse was adequate, viz 72%.

Mepivacaine plasma concentrations associated with the onset of all toxic manifestations were similar between both groups (table 2). Convulsions began at 17.4 \pm 1.7 and 19.5 \pm 0.7 μ g/ml in nonpregnant and pregnant animals, respectively. The corresponding concentrations at circulatory collapse were 41.6 \pm 2.4 and 45.6 \pm 6.2 μ g/ml. The CC/CNS ratio of plasma mepivacaine concentrations were not significantly different between the two groups.

Mean concentrations of mepivacaine in the brain were

TABLE 3. Mean (\pm SE) of Heart Rate and Mean Arterial Blood Pressure, before Infusion, and at Each Toxic Manifestation

	Control	Convulsions	Hypotension	Apnea
Nonpregnant				
Heart rate (bpm)	115 \pm 11	209 \pm 17*	143 \pm 18	113 \pm 18
Mean arterial pressure (mmHg)	93 \pm 2	175 \pm 8*	74 \pm 9*	40 \pm 5*
Pregnant				
Heart rate (bpm)	94 \pm 9	193 \pm 15*	142 \pm 17*	110 \pm 19
Mean arterial pressure (mmHg)	92 \pm 6	151 \pm 7*	57 \pm 10*	25 \pm 5*

* Significantly different from control ($P < 0.05$).

TABLE 4. pH_a, Pa_{CO₂}, and Pa_{O₂} in Nonpregnant and Pregnant Ewes during Mepivacaine Infusion (Mean \pm SE)

	Control	Convulsions	Hypotension	Apnea	Circulatory Collapse
Nonpregnant					
pH _a	7.47 \pm 0.02	7.48 \pm 0.02	7.27 \pm 0.02*	7.24 \pm 0.03*	7.22 \pm 0.02*
Pa _{CO₂} (mmHg)	32 \pm 2	31 \pm 2	49 \pm 3*	52 \pm 2*	55 \pm 2*
Pa _{O₂} (mmHg)	90 \pm 9	88 \pm 5	36 \pm 4*	33 \pm 6*	26 \pm 3*
Pregnant					
pH _a	7.46 \pm 0.05	7.44 \pm 0.04	7.31 \pm 0.05*	7.28 \pm 0.02*	7.27 \pm 0.03*
Pa _{CO₂} (mmHg)	34 \pm 1	35 \pm 2	45 \pm 4*	49 \pm 2*	50 \pm 3*
Pa _{O₂} (mmHg)	101 \pm 1	84 \pm 3*	48 \pm 8*	37 \pm 4*	35 \pm 3*

* Significantly different from control ($P < 0.05$).

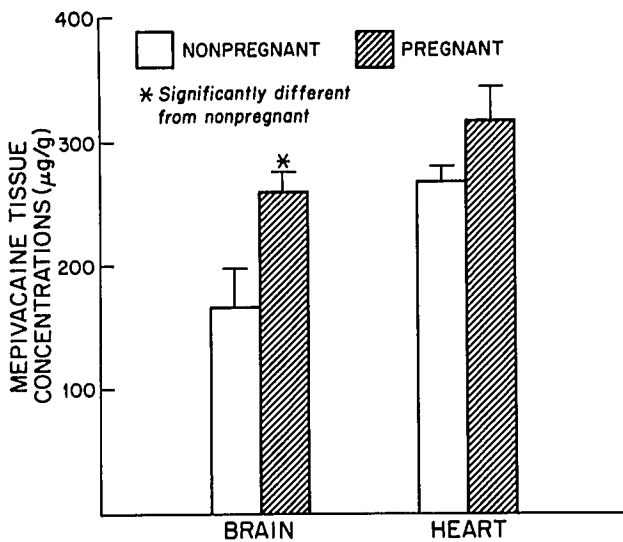


FIG. 1. Mean (\pm SE) mepivacaine concentrations in brain and heart of nonpregnant ($N = 7$) and pregnant ewes ($N = 6$) ($P < 0.05$).

lower in the nonpregnant animals, 174.4 ± 22.3 , versus 258.7 ± 14.4 $\mu\text{g/g}$ wet weight in the pregnant animals (fig. 1). In the heart, they were not significantly different, 268.1 ± 12.3 and 317.0 ± 26.2 $\mu\text{g/g}$.

No malignant ventricular arrhythmias were observed before circulatory collapse.

The mean drug concentrations in sera tested for protein binding were similar between nonpregnant and pregnant animals. For mepivacaine, these were 8.5 ± 0.2 , 16.5 ± 0.7 , and 49.2 ± 1.4 $\mu\text{g/ml}$ for the nonpregnant, and 8.5 ± 0.2 , 17.0 ± 0.2 , and 53.5 ± 0.9 $\mu\text{g/ml}$ in the

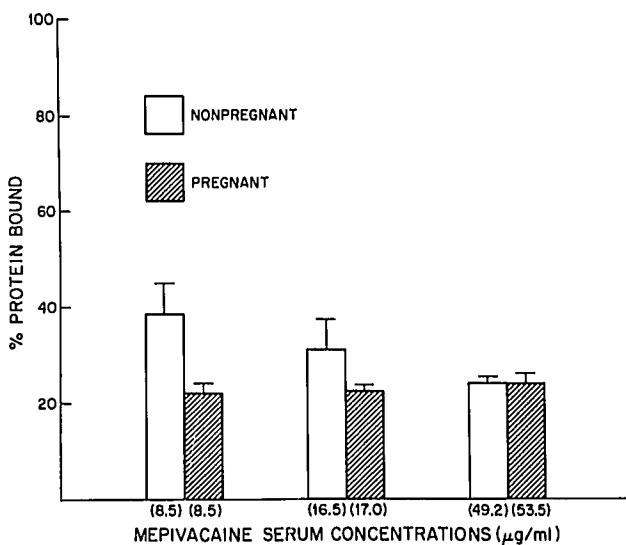


FIG. 2. Mean (\pm SE) percentage of mepivacaine bound to serum proteins in nonpregnant and pregnant ewes at different serum concentrations ($P < 0.0166$).

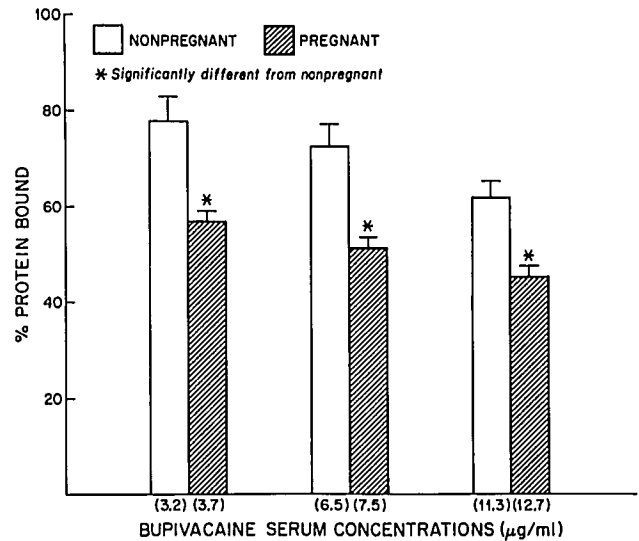


FIG. 3. Mean (\pm SE) percentage of bupivacaine bound to serum proteins in nonpregnant and pregnant ewes at different serum concentrations ($P < 0.0166$).

pregnant. Bupivacaine concentrations tested were 3.2 ± 0.1 , 6.5 ± 0.2 , and 11.3 ± 0.4 $\mu\text{g/ml}$ in the nonpregnant, and for the pregnant, 3.7 ± 0.1 , 7.5 ± 0.4 , and 12.7 ± 0.3 $\mu\text{g/ml}$.

The percentage of drug bound to serum proteins is represented in figures 2 and 3. There was no significant difference in the proportion of mepivacaine bound to proteins between pregnant and nonpregnant animals, at all concentrations tested (fig. 2), although at the lowest concentration there was a tendency toward lower protein binding in sera obtained from pregnant ewes ($P = 0.04$). In contrast, significantly lower binding was found for bupivacaine in pregnant sheep for all three drug concentrations (fig. 3).

Discussion

These data indicate that pregnant ewes are no more sensitive to the cardiotoxicity of mepivacaine than are nonpregnant ewes, since the lethal doses and plasma concentrations were similar in both groups. This is in contrast to our finding that pregnancy enhances the cardiotoxicity of bupivacaine,³ and may be partially explained by the pregnancy-induced differences in protein binding for these two drugs.

In this study, the serum protein binding of mepivacaine was not lower in pregnant animals at all concentrations studied. However, at the lowest mepivacaine concentration, there was a tendency toward lesser protein binding in the pregnant group. Statistical significance might have been reached if the power of analysis were enhanced by a larger number of determinations. The most important differences between mepivacaine and bupivacaine were

seen at drug concentration associated with fatal toxic symptoms. At cardiovascular collapse, the proportion of bound mepivacaine was approximately 22% in both pregnant and nonpregnant animals. In contrast, the proportion of bound bupivacaine was lower in pregnant ewes, being 51%, compared to 73% in the nonpregnant, at serum concentrations approximating those previously found at cardiac arrest.³ Therefore, while lethal total concentrations of bupivacaine (previously determined) in nonpregnant and pregnant animals were significantly different, namely 8.0 and 5.5 $\mu\text{g/ml}$, concentrations of free drug were similar, 2.2 and 2.7 $\mu\text{g/ml}$, respectively. Thus, the difference between the two drugs may be related to gestational increases in the availability of free bupivacaine, but not of mepivacaine. However, enhanced sensitivity of the myocardium to bupivacaine during pregnancy should also be considered. In a recent, *in vitro* study, a more profound electrophysiological effect of bupivacaine, but not lidocaine, was shown in the Purkinje fibers and ventricular muscle cells of progesterone treated *versus* nontreated rabbits.¹³

Serum protein binding experiments were conducted under controlled conditions to avoid artifacts. While it would have been preferable to draw blood from the animals actually involved in the toxicity study the risk of hypovolemia and enhanced cardiovascular toxicity made this method less desirable. Furthermore, it would have been a deviation from the protocol followed formerly in the bupivacaine toxicity study. Instead, sera were collected from separate animals at the breeder's farm. Studies were conducted on several batches of pooled serum.

The determination in *pH* and blood gas values followed a similar pattern in both groups of animals, while the observation that P_{O_2} had already dropped significantly at the onset of convulsions in the pregnant sheep was probably due to increased oxygen consumption associated with pregnancy.

Myocardial concentrations of mepivacaine at circulatory collapse were also similar in pregnant and nonpregnant animals, as was the case for bupivacaine.³ Although brain concentrations of mepivacaine at this time were higher in the pregnant ewes, those associated with the onset of convulsions could not be determined. Bupivacaine concentrations at cardiovascular collapse were higher in the brain of nonpregnant animals. The reason for this difference between the two drugs in this instance is not clear. Another difference was the absence of malignant cardiac arrhythmias, which were a prevalent feature of bupivacaine toxicity.

In nonpregnant and pregnant animals, comparison of cardiac collapse to convulsive dose and plasma concentration ratios (CC/CNS) between mepivacaine and our published data on bupivacaine³ indicate that the margin of safety for mepivacaine is greater (table 5).

TABLE 5. Mean (\pm SE) Ratios of Circulatory Collapse (CC) and Convulsive (CNS) Dosages and Plasma Concentrations of Local Anesthetics in Nonpregnant and Pregnant Ewes

CC/CNS	Mepivacaine	Bupivacaine
Nonpregnant ewes		
Dosage ratio	7.6 \pm 0.4*	3.7 \pm 0.5
Plasma concentration ratios	2.5 \pm 0.2*	1.6 \pm 0.1
Pregnant ewes		
Dosage ratios	9.2 \pm 1.3*	2.7 \pm 0.4
Plasma concentration ratios	2.4 \pm 0.3*	1.4 \pm 0.1

* Significantly different from bupivacaine ($P < 0.05$).

In conclusion, our data indicate that mepivacaine cardiotoxicity, unlike that of bupivacaine, is not enhanced by pregnancy. Furthermore, our data suggest that the difference between the two drugs may be related to pregnancy-associated increases in the availability of free bupivacaine, but not mepivacaine.

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