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Effects of Progesterone on the Cardiac Electrophysiologic Action of Bupivacaine and Lidocaine

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Pregnancy is accompanied by an increased cardiac and neural sensitivity to some local anesthetic agents such as bupivacaine. The current study was initiated to investigate the relationship between increased progesterone concentrations and the electrophysiologic effects of bupivacaine, and lidocaine in isolated Purkinje fiber (PF)-ventricular muscle (VM) preparations. Twenty-four oophorectomized female white rabbits were killed after receiving 30 $mg \cdot kg^{-1} \cdot day^{-1}$ of progesterone intramuscularly or peanut oil alone for 4 days. PF and VM action potentials were recorded using standard electrophysiologic procedures. Plasma progesterone concentrations were 5 ± 2.9 ng/ml in control animals compared to 59.8 ± 11.0 ng/ml in progesterone-treated animals ($P < 0.05$). Bupivacaine (3.5 − 17.4 μM) depressed the maximal rate of depolarization (V_{max}) of PF to a significantly greater extent in tissues from progesterone-treated animals as compared to control animals. For example, at 3.5 μM bupivacaine decreased PF V_{max} 52% in progesterone-treated tissues compared to 32% in controls ($P < 0.05$); the V_{max} of VM was also depressed to a greater extent in tissues from progesterone-treated animals ($P < 0.001$). Lidocaine did not demonstrate an enhanced depressant effect in tissues from progesterone-treated animals. These results indicate that progesterone selectively increases the cardiac membrane depressant effects of bupivacaine but not lidocaine. This may contribute to the enhanced toxicity of bupivacaine in pregnant animals. (Key words: Anesthetics, local: bupivacaine; lidocaine. Heart: action potential; maximal rate of depolarization. Steroid: progesterone.)

THE EFFECTS of local anesthetic agents on peripheral nerves[1,2] appear to be enhanced during pregnancy. A similar enhancement specifically for bupivacaine is observed on the cardiovascular system during pregnancy.[3] For example, the local anesthetic requirements for a similar degree of epidural blockade are less in parturients than in their nonpregnant counterparts. This is not related exclusively to physical or mechanical factors.[4] Isolated nerve studies have demonstrated that bupivacaine caused a faster onset of conduction block and a more profound degree of block in nerves from pregnant animals as compared to nonpregnant animals.[1,2] Moreover, the intramuscular administration of progesterone was found to increase the sensitivity of isolated nerves to local anesthetic-induced conduction block.¶

With regard to the cardiovascular effects of local anesthetics, a smaller dose of bupivacaine was required to induce cardiovascular collapse in pregnant ewes compared to comparable nonpregnant animals.[3] On the other hand, the doses of lidocaine and mepivacaine required to produce cardiovascular collapse were similar in pregnant and nonpregnant ewes.[5,6] Mendoza and DeMello[7] reported direct membrane effects of progesterone (10^{-8}–10^{-7} M) on the membrane potential of guinea pig myocardial fibers. Effects included hyperpolarizing the membrane (7 mV) and increasing the maximal rate of depolarization (V_{max}). The current study determined the effects of bupivacaine or lidocaine concentrations, that might be obtained after an accidental intravenous injection, on the electrophysiologic properties of cardiac tissues previously exposed to elevated progesterone concentrations. Baseline data were obtained in control animals with normal progesterone concentrations. The results suggest that the enhanced cardiac toxicity of bupivacaine in pregnant animals may be related to an increased progesterone concentration.

Materials and Methods

All animals were housed, cared for, and killed in accordance with National Institutes of Health guidelines. This study was approved by the Harvard Medical Area Standing Committee on Animals.

Twenty-four female white New Zealand rabbits were oophorectomized while anesthetized with halothane. The animals were allowed to recover from the surgery for 2 weeks. Then they were randomized into two groups, one of which received progesterone ($30\ mg \cdot kg^{-1} \cdot day^{-1}$ for 4 days; n = 14) and the other of which received the same volume of the peanut oil vehicle (n = 10). On the 5th day, the animals were anesthetized with thiamylal ($20\ mg \cdot kg^{-1}$) and then killed by air embolus injection. A blood sample was taken at the time of killing for determination of progesterone concentrations by radioimmu-

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¶ Flanagan HL, Datta S, Moller RA, Covino BG: Effects of exogenous progesterone on bupivacaine-induced conduction blockade in the isolated rabbit vagus nerve (abstract). ANESTHESIOLOGY 69:A676, 1988.

noassay with antiserum from rabbits using ^{3}H-progesterone.[8] The heart was quickly removed and placed in a warm aerated Tyrode's solution. The composition of the Tyrode's solution was (millimolar): NaCl 137, KCl 4.0, $CaCl_2$ 1.8, $MgCl_2$ 0.9, $NaHCO_3$ 25, NaH_2PO_4 1.2, and glucose 11. The solution was aerated with 95% O_2/5% CO_2, warmed to 37° C, and maintained at a *p*H of about 7.35.

The right septal wall was dissected out and placed in a recording chamber where extracellular stimulating electrodes were placed on the proximal right bundle branch and intracellular recording electrodes (filled with 3 M KCl) were inserted into the distal bundle branch and myocardium, as previously described.[9] Action potentials were evoked by rectangular pulses 1 ms in duration at a frequency of 2 Hz. The voltage intensity was twice that required to evoke a response during diastole (2 × threshold). Single-cell impalements were attempted throughout these experiments. If impalements were lost and impalements resulting in the same electrophysiologic values could not be attained, the preparations were discarded.

After 1 h of equilibration in progesterone-free Tyrode's solution, superfusion with one of the local anesthetics was begun. Half of the tissues from the control and progesterone-treated animals were exposed to each local anesthetic. Local anesthetic concentrations were increased every 30 min. Bupivacaine concentrations of 3.5, 10.4, and 17.4 μM (1, 3, and 5 μg/ml; n = 12: 7 progesterone and 5 peanut oil) and lidocaine concentrations of 21, 43, and 85 μM (5, 10, and 20 μg/ml; n = 12 as above) were used. The following electrophysiologic parameters were recorded: maximum diastolic potential, action potential amplitude, V_{max}, action potential duration measured at 50% of repolarization (APD_{50}), and conduction time. Measurements were made after 1 h equilibration in drug-free Tyrode's and after 30 min exposure to the various concentrations of bupivacaine and lidocaine. After 30 min exposure to the highest concentrations of either drug, the tissues were exposed to drug-free Tyrode's for 1 h. During this time, V_{max} was measured at 5-min intervals for 30 min and then every 15 min to determine when it returned to 80% of the control values.

The concentrations of bupivacaine and lidocaine used in this study were high considering there was no protein binding in the perfusion solution. However, as reported previously by our group, these concentrations are similar to those attained in some animal experiments[9] after intravenous injections. Bupivacaine and lidocaine concentrations of 30 and 100 μg/ml, respectively, have been measured in laboratory animals after an intravenous injection. In these studies, protein binding would be at the 50% level for bupivacaine and would result in a free bupivacaine concentration of about 15 μg/ml. This concentration of bupivacaine would completely depress the excitability of the Purkinje fiber (PF) and ventricular muscle (VM) tissues. Therefore, we chose to use lower concentrations of 1, 3, and 5 μg/ml of bupivacaine. Bupivacaine and lidocaine were kindly supplied by Astra Alab (Sodertalje, Sweden).

Data were analyzed using an analysis of variance and Tukey's honestly significant difference test. Some data were analyzed using the chi-square test. A P level of 0.05 was considered statistically significant.

Results

The average blood concentration of progesterone in the peanut oil–treated group was 5.0 ± 2.9 ng/ml, compared to a concentration of 59.8 ± 11.0 ng/ml in the progesterone-treated group. There were no significant differences between the groups with respect to the baseline values of the various electrophysiologic parameters that were monitored.

The maximum diastolic potential of the PF action potentials was not significantly altered by either bupivacaine or lidocaine in the hearts from progesterone-treated rabbits or controls (table 1). At bupivacaine concentrations of 10.4 μM and greater the number of preparations decreased in both groups. In those preparations the applied voltage (2 × threshold) was unable to evoke PF action potentials in a 1:1 manner at a frequency of 2 Hz. A local anesthetic–induced decrease in PF action potential amplitude was observed in both bupivacaine groups of tissues (table 1). A significant decrease in action potential amplitude was achieved at a bupivacaine concentration of 10.4 μM in PF from progesterone-treated animals. At 10.4 μM bupivacaine, inexcitability was induced in five progesterone pretreated tissues and two control tissues (table 1). This reduced the numbers in the other electrophysiologic parameters except maximum diastolic potential. Lidocaine did not significantly affect action potential amplitude in either group at any concentration.

A concentration-dependent decrease in V_{max} was observed in bupivacaine-treated PF from both groups (table 2). However, a significantly greater depression of V_{max} occurred at 3.5 and 10.4 μM of bupivacaine in PF from progesterone-treated animals as compared to the peanut oil–treated group. For example, in the control group 3.5 μM of bupivacaine decreased V_{max} by 32%, which was significantly less than the 52% decrease in V_{max} observed in the progesterone group ($P < 0.001$). At 17.4 μM of bupivacaine, V_{max} was depressed by approximately 78% in the progesterone (n = 1) and control (n = 2) groups. Lidocaine significantly decreased V_{max} at 85.4 μM only in the progesterone-treated tissues.

Bupivacaine also significantly depressed V_{max} in VM from both groups beginning at 3.5 μM ($P < 0.05$). However, the decrease was significantly greater in the pro-

TABLE 1. Local Anesthetic Effects on Various Purkinje Fiber Action Potential Parameters

Local Anesthetic Concentration (μM)	Bupivacaine/ Progesterone	Bupivacaine/ Placebo	Lidocaine/ Progesterone	Lidocaine/ Placebo
Maximum diastolic potential (−mV)				
Baseline	86.6 ± 2.9	92 ± 1.3	89.7 ± 2.1	83.3 ± 1.9
3.5	84.0 ± 2.9	91.2 ± 2.3		
10.4	79 ± 1.0 (n = 2)	90.7 ± 2.7		
17.4	84 (n = 1)	90.0 ± 6.0		
21.4			88 ± 2.2	86.0 ± 1.7
42.7			89.7 ± 2.7	85.0 ± 0.9
85.4			90.4 ± 2.7	85.3 ± 1.8
Wash	88.5 ± 2.6	87 ± 3.0	95 ± 1.5	89.2 ± 1.0
Action potential amplitude (mV)				
Baseline	119.7 ± 3.1	120.8 ± 3.2	125.4 ± 2.6	118.0 ± 4.1
3.5	110.3 ± 2.8	115.6 ± 2.8		
10.4	91 ± 5.0* (n = 2)	108 ± 4.2 (n = 3)		
17.4	94 (n = 1)	98 ± 16 (n = 2)		
21.4			126 ± 2.8	119.3 ± 3.8
42.7			123.6 ± 1.7 (n = 5)	114.7 ± 6.8
85.4			123 ± 3.1 (n = 4)	115.6 ± 2.9
Wash	121 ± 2.6	109 ± 1.0	124 ± 3.6	122 ± 4.6

Values are mean ± SEM. n = 7 for progesterone treatment; n = 5 for placebo treatment.

* $P < 0.05$ compared to baseline.

TABLE 2. Local Anesthetic Effects on Action Potential Maximal Rate of Depolarization

Local Anesthetic Concentration (μM)	Bupivacaine/ Progesterone	Bupivacaine/ Placebo	Lidocaine/ Progesterone	Lidocaine/ Placebo
V_{max} in Purkinje fibers (V/s)				
Baseline	577 ± 57	606 ± 49	610 ± 19	583 ± 21
3.5	287 ± 40*†	410 ± 29*		
10.4	175 ± 25*‡ (n = 2)	280 ± 12* (n = 3)		
17.4	100* (n = 1)	150 ± 100§ (n = 2)		
21.4			582 ± 36	568 ± 31
42.7			530 ± 24	487 ± 69
85.4			424 ± 39§	434 ± 50
Wash	472 ± 63	544 ± 50	648 ± 15	580 ± 13
V_{max} in ventricular muscle (V/s)				
Baseline	141 ± 15	162 ± 29	157 ± 21	190 ± 27
3.5	66 ± 10†§	124 ± 16		
10.4	n = 0	90 ± 10 (n = 2)		
17.4	n = 0	80 (n = 1)		
21.4			131 ± 20	123 ± 16
42.7			133 ± 34	123 ± 21
85.4			120 ± 41	102 ± 17
Wash	118 ± 15	150 ± 35	162 ± 35	136 ± 22

Values are mean ± SEM. n = 7 for progesterone treatment; n = 5 for placebo treatment.

* $P < 0.05$ compared to baseline.

† $P < 0.001$ compared to bupivacaine/placebo.

‡ $P < 0.05$ compared to bupivacaine/placebo.

§ $P < 0.001$ compared to baseline.

TABLE 3. Local Anesthetic Effects on Various Action Potential Parameters

Local Anesthetic Concentration (μM)	Bupivacaine/ Progesterone	Bupivacaine/ Placebo	Lidocaine/ Progesterone	Lidocaine/Placebo
Purkinje fiber APD_{50} (ms)				
Baseline	162.9 ± 9.8	159.8 ± 8.7	157.4 ± 8.9	154.8 ± 9.7
3.5	142.9 ± 2.1	143.2 ± 11.3		
10.4	129 ± 15 (n = 2)	131.3 ± 5.9 (n = 3)		
17.4	160 (n = 1)	127 ± 0 (n = 2)		
21.4			122.6 ± 5.5*	129.7 ± 5.2
42.7			121.8 ± 6.0*	126 ± 7.5
85.4			121 ± 6.5*	123.4 ± 6.3*
Wash	166 ± 12	106	154.8 ± 9.9	155.0 ± 7.1
Conduction time normalized (%)				
Baseline	100	100	100	100
3.5	301 ± 86	186 ± 46		
10.4	(n = 0)	324 ± 10 (n = 2)		
17.4	(n = 0)	369 (n = 1)		
21.4			128 ± 25	109 ± 10
42.7			154 ± 24	141 ± 28
85.4			185 ± 21 (n = 4)	149 ± 53
Wash	133 ± 27	144	94 ± 12	108 ± 16

Values are mean ± SEM. n = 7 for progesterone treatment; n = 5 for placebo treatment. Conduction time was calculated as the time between the V_{max} of the ventricular muscle and the Purkinje fiber action potentials.

* $P < 0.05$ compared to baseline.

gesterone-treated group than in the control group ($P < 0.001$). Lidocaine-induced decreases in V_{max} of VM were similar in both groups.

Bupivacaine produced slight yet nonsignificant decreases in APD_{50} (table 3). Lidocaine significantly decreased APD_{50} at 21 μM in the progesterone-treated tissues ($P < 0.05$); increasing lidocaine's concentration to 85.4 μM produced no further change in APD_{50}. In the control group, lidocaine significantly decreased APD_{50} only at 85.4 μM. Neither local anesthetic produced significant differences in APD_{50} between the progesterone and peanut oil–treated groups.

Conduction time between the PF and VM tissues was prolonged in preparations exposed to bupivacaine. All tissues from progesterone-treated animals showed complete PF–VM conduction block at 10.4–17.4 μM bupivacaine. This was significantly greater, using chi-square analysis, than in the control group, which showed complete PF–VM conduction block in three of five preparations at 10.4 μM and four of five preparations at 17.4 μM. Lidocaine prolonged conduction time in both groups to a smaller extent. However, 43% of the preparations in the progesterone-treated group showed complete conduction block at 85.4 μM compared to 0% in the peanut oil–treated group (table 3).

Discussion

Previous studies have demonstrated that bupivacaine causes significantly greater depression of various cardiac electrophysiologic parameters compared to lidocaine.[9] These differences are perhaps responsible for the enhanced cardiac toxicity of bupivacaine in animals as compared to lidocaine.[10–12] Comparative studies in pregnant and nonpregnant sheep have also demonstrated that bupivacaine but not lidocaine or mepivacaine causes cardiovascular collapse at lower doses in pregnant animals.[3,5,6] It is not certain whether this enhanced toxicity of bupivacaine in pregnant animals is due to a greater sensitivity of the cardiac tissue to this agent or to alterations in pharmacokinetic properties of the drug, such as decreased plasma protein binding.**

Isolated nerve studies have demonstrated an enhanced sensitivity to bupivacaine-induced conduction block in nerves from pregnant animals or animals exposed to high progesterone blood levels.[2,¶] Attempts to carry out studies in isolated cardiac tissues from pregnant animals has

** Santos A, Pedersen H, Finster M, Morishima H, Arthur G: Serum protein binding and cardiotoxicity of bupivacaine and mepivacaine (abstract). ANESTHESIOLOGY 67:A249, 1987.

proven difficult because of the variability in tissue response. In the current study, progesterone blood concentrations were increased by intramuscular administration in order to mimic the pregnant state in rabbits. The progesterone concentrations that were attained were higher than those observed in pregnant rabbits,[2] although they are similar to those measured in humans. However, gestation in rabbits is 28 days long. Because we planned to administer progesterone for only 4 days, we chose to use the higher progesterone concentrations to induce these changes in this short time. The results clearly demonstrate that progesterone can enhance the myocardial depressant effects of bupivacaine but not lidocaine, with a bupivacaine mean effective concentration (EC_{50}) of 0.8 μM for depression of V_{max}. This concentration is well within the range achievable during some regional anesthetic procedures. This confirms the differential cardiac toxicity of bupivacaine but not lidocaine in pregnant and nonpregnant sheep[3,6] and also suggests that the enhanced sensitivity in pregnancy may be related to an exaggerated direct myocardial action of bupivacaine rather than a change in pharmacokinetic properties of the agent. The data also suggest that the increase in progesterone levels during pregnancy may be responsible for this enhanced sensitivity.

The etiology of this increased bupivacaine cardiac depression in the presence of progesterone is not clear. One of the differences between bupivacaine and lidocaine at the sodium channel resides in the binding kinetics to the receptor. Bupivacaine's dissociation constant (K_d) is 10 times greater than that for lidocaine.[13] This means that bupivacaine remains bound to the sodium channel longer in the inactivated state. The possibility also exists that bupivacaine could be binding intracellularly to a receptor site for which it competes with progesterone. As the progesterone concentration increases intracellularly, the unbound bupivacaine concentration also increases, thus presenting the sodium channel with a greater number of bupivacaine molecules with which to bind. On the other hand, progesterone may affect the state of the sodium channel or the binding to the receptor site directly.

In addition, progesterone alters certain electrophysiologic properties of cardiac membranes.[7] However, the effects reported would tend to oppose the depressant effects of bupivacaine rather than enhance them. In heart cell culture, progesterone increased the arrhythmogenicity of bupivacaine by decreasing the arrhythmic concentration by 50%.†† This increased arrhythmogenic effect is similar to what we have seen in the present study: the previous results indicated that the normal rate of spontaneously beating cells was decreased with intermittent block of action potentials similar to the block of action potentials that we had at 10.4 μM bupivacaine. Finally, it is not clear why progesterone should affect the action of bupivacaine but not of lidocaine.

Clearly, further studies involving acute application of progesterone, β estradiol, and other sex steroids to isolated cardiac tissues for microelectrode studies or even isolated myocytes for patch clamp studies are required to delineate further the mechanism of action of progesterone for increasing myocardial sensitivity to bupivacaine.

This paper is dedicated to the memory of Dr. Benjamin G. Covino, clinician, scientist, administrator, colleague, and friend.

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