Pregnancy Does Not Increase Susceptibility to Bupivacaine in Spinal Root Axons

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Background: The underlying mechanism of enhanced antinociceptive effects and increased susceptibility to local anesthetics during pregnancy is not known. Mechanical, hormonal, biochemical, and neural changes have been suggested. The authors measured the susceptibility of individual spinal root axons to bupivacaine during late pregnancy in rats and compared them with similar measurements in nonpregnant rats.

Methods: Lumbar dorsal and ventral roots were excised from anesthetized pregnant and nonpregnant rats. Single-fiber dissection and recording techniques were used to isolate activity in individual axons. Supramaximal constant voltage stimuli were delivered to the distal end of the root. During in vitro perfusion, each root was exposed to increasing concentrations of bupivacaine, and the minimum blocking concentration (Cₐ) and the concentration that increased conduction latency by 50% (EC₅₀) were measured.

Results: Myelinated and unmyelinated dorsal and ventral root axons of pregnant rats appeared to be less sensitive to steady-state conduction block and to the latency-increasing effects of bupivacaine than were equivalent axons from nonpregnant rats. Although when comparing specific axon types, only the difference in Cₐ was significant (Cₐ = 29.8 μM for pregnant and Cₐ = 22.1 μM for nonpregnant rats, P < 0.05; EC₅₀ = 19.9 μM and 13.6 μM, respectively).

Conclusions: In contrast to clinical expectations, the susceptibility to bupivacaine conduction block in individual dorsal and ventral root axons during late pregnancy in rats was not greater in pregnant animals. Pregnancy-related changes in diffusion barriers and activation of endogenous analgesic systems without changes in the electrophysiologic properties of spinal root axons are suggested as possible explanations for the discrepancy between clinical and experimental observations. (Key words: Bupivacaine. Conduction block; Dorsal root axons. Local anesthetics. Pregnancy. Rat. Ventral root axons.)

PREGNANCY increases women’s sensitivity to general and local anesthetics. Clinical investigations have shown that women usually require smaller doses of local anesthetics for epidural anesthesia during pregnancy.1-3 These clinical observations were confirmed in a recent laboratory study that showed that the analgesic effect of epidural lidocaine in rats was significantly enhanced during pregnancy.4 The mechanism by which pregnancy causes an increased susceptibility to local anesthetics is unknown. Mechanical, hormonal, and biochemical changes have been suggested. For example, the increased levels of progesterone and endorphins that occur during pregnancy may contribute directly or through synergistic mechanisms to increase local anesthetic effectiveness. Progesterone has been shown to potentiate the analgesic effect of intrathecally administered sufentanil5 and may augment local anesthetic effects by acting on endogenous opiates or their receptors. In addition, chronic but not acute exposure to progesterone has been shown to increase local anesthetic sensitivity in rabbit vagus nerve fibers.6-8 Gestational exposure to high levels of progesterone may alter perineural structures to increase drug permeability through the nerve sheath, or it may induce changes in nerve membrane structure that enhance sensitivity to local anesthetics. Peripheral nerve studies in humans9 and in rabbits9,10 have concluded that peripheral nerves are more sensitive to the effects of local anesthetics during pregnancy, suggesting that similar changes in spinal nerves may account for decreased local anesthetic requirements for spinal and epidural analgesia or anesthesia.

Among the local anesthetics used for regional anesthe-
sia in obstetrics, bupivacaine at low concentrations (e.g., 2–4 mM or 0.0625–0.125%) can provide excellent sensory anesthesia with minimal impairment of motor function. This differential sensory nerve block has been shown to exist in pregnant and nonpregnant patients. Interestingly, when we compared the local anesthetic sensitivity of motor and sensory spinal root axons in male rats, motor fibers were blocked at significantly lower concentrations of bupivacaine than were sensory nerve fibers. This finding shows that dorsal root (DR) axons are not intrinsically more sensitive to local anesthetics and suggests that pregnancy-induced changes in nerve structure or function may account for the clinically observed differential block.

In the present study, we tested the hypothesis that pregnancy-induced changes in spinal root axons account for the enhanced susceptibility to local anesthetics observed clinically. Specifically, we measured the susceptibility of individual dorsal and ventral root (VR) axons to bupivacaine during late pregnancy in rats and compared them with similar measurements in nonpregnant rats.

Materials and Methods

Approval was obtained from the Stanford University Administrative Panel on Laboratory Animal Care. Six time-mated pregnant Sprague-Dawley rats weighing 280–400 g on day 21 of their pregnancy and six adult age-matched female Sprague-Dawley rats weighing 180–220 g were anesthetized with enflurane (1–2.5%) and nitrous oxide (70%) in oxygen. As previously described,11,12 the spinal cord was exposed through a thoracolumbar laminectomy; using microsurgical techniques, single lumbar dorsal and VRs were excised and transferred to a perfusion-recording chamber. The roots were continuously superfused with artificial cerebrospinal fluid (Na+, 150 mM; K+, 4 mM; Cl−, 127 mM; Ca2+, 2 mM; Mg2+, 1.3 mM; PO43−, 1.2 mM; HCO3−, 26 mM; glucose, 11 mm) at 37 ± 0.5°C and equilibrated with 95% oxygen and 5% carbon dioxide gas mixture to maintain a pH of 7.3–7.4. Single-fiber microdissection and recording techniques were used to isolate activity in individual spinal root axons.

Supramaximal constant-voltage stimuli at 0.3 Hz (lasting 0.1 ms) were delivered to the distal end of the isolated root. Single-fiber action potentials were amplified and displayed on a digital storage oscilloscope and recorded for computer analysis. Stimulus-evoked activity in individual axons was monitored and the latency between the stimulus and action potential was measured. Conduction velocity (CV) was calculated from measurements of conduction latency and length of axon between stimulating and recording electrodes (CV [m/s] = conduction distance [mm] divided by conduction latency [ms]).

After 20–30 min of control measurements, each nerve root was exposed in a stepwise manner to increasing concentrations of bupivacaine. Conduction latency was measured at 1-min intervals until the unit failed or until the CV remained constant for three consecutive measurements (usually <10 min). Steady-state conditions were reached before increasing the bupivacaine concentration (in 2–6 μM increments) to the next level. The minimum blocking concentration (C50) of bupivacaine was the lowest concentration that resulted in conduction failure. Only one incremental series of measurements was obtained from each nerve root, and data were accepted only from those axons that recovered completely after return to control artificial cerebrospinal fluid. For each axon, the EC50 for bupivacaine was calculated from the slope of the line relating bupivacaine concentration to conduction latency at the point representing a 50% latency increase (fig. 1).

For data analysis, DR axons were divided into two groups based on their conduction velocity characteristics. Those DR axons with a CV greater than 3 m/s were considered to be myelinated (A-fiber), whereas those with a CV less than 1.5 m/s were presumed to be unmyelinated (C-fiber). Ventral root axons all had CVs greater than 3 m/s, comparable to the DR-A-fiber group. Results are presented as means ± SEM when appropriate. For normally distributed data, differences between groups of equal variance were tested using Student’s t tests, but otherwise the Mann-Whitney Rank Sum test was used to determine statistical differences. Differences were considered significant at P < 0.05.

Results

Sensitivity to bupivacaine-induced conduction block and the conduction velocity slowing effects of bupivacaine were measured in 50 DR and 8 VR axons from 6 full-term pregnant and 6 nonpregnant rats. The length of axons exposed to bupivacaine varied from 16–28 mm for pregnant (mean, 21.6 mm) and from

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17–23 mm for nonpregnant rats (mean, 20.5 mm). The minimum bupivacaine $C_m$ for all DR axons ranged from 16–75.2 μM (fig. 2). As shown in figure 3, the mean minimum bupivacaine $C_m$s for myelinated DR axons (14 ± 4.2 μM), unmyelinated DR axons (29.8 ± 2.4 μM), and VR axons (27.8 ± 5.7 μM) excised from pregnant animals were higher than the comparable $C_m$s from nonpregnant animals by 27%, 35%, and 55%, respectively. Statistical comparisons between pregnant and nonpregnant axon groups showed that only C-fibers from pregnant rats were significantly less sensitive to the conduction blocking effects of bupivacaine ($P < 0.05$). However, combining probabilities from tests of significance\cite{16} for all three axon groups statistically supports the conclusion that pregnancy decreases the susceptibility of spinal root axons to the conduction blocking effect of bupivacaine ($P < 0.05$).

Because the latency increasing (CV slowing) effect of sodium channel blockade is another measure of axon sensitivity to local anesthetics, steady-state lat-
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Fig. 4. Bupivacaine concentrations producing a 50% increase in conduction latency. Values are mean ± SEM. Significant differences (P < 0.02) between pregnant and nonpregnant rat axon groups are indicated by *.

Consistent with previous observations in male rats, a weak negative relation between conduction velocity (which is a measure of axon diameter) and sensitivity to bupivacaine could be found by linear regression analysis (R² = 0.18 for pregnant and R² = 0.3 for nonpregnant rat axons). Comparison of these regression lines for pregnant and nonpregnant rats using a multiple linear regression procedure did not reveal any statistically significant difference (P = 0.136).

Discussion

This is the first study to directly examine the local anesthetic sensitivity of mammalian dorsal and ventral root axons during pregnancy. Contrary to clinical expectations, the results of this study clearly show that spinal root axons from full-term pregnant rats are not more susceptible to the anesthetic effect of bupivacaine when compared with nonpregnant controls. It has been shown clinically and experimentally that during pregnancy dose requirements of local anesthetics are reduced in epidural anesthesia, and sensitivity to anesthetic effects is increased in peripheral and central nerve blocks. Several mechanisms for the increased susceptibility to local anesthetics during pregnancy have been proposed. An enhanced spread of epidurally administered local anesthetic may be caused mechanically by increased pressure in the epidural space (e.g., as a consequence of uterine contractions), or by distension of the epidural veins as the result of partial obstruction of the inferior vena cava by the gravid uterus. This explanation is inadequate, however, because a significantly facilitated spread of epidural anesthesia has been reported during the first trimester of pregnancy, when contractions and mechanical factors related to the gravid uterus are unlikely to play important roles. Mechanical factors cannot be used to explain reports of increased sensitivity to lidocaine in median nerve block in pregnant women or in sciatic nerve block in pregnant rats. Finally, enhanced local anesthetic effects have been reported in isolated peripheral nerves when all mechanical factors have been excluded.

As an alternative explanation, the increase in effectiveness of local anesthetics during pregnancy may be mediated by the endorphin system, as suggested by Gintzler, who observed increased pain thresholds in pregnant rats compared with nonpregnant rats. In contrast to that study, Dahl et al. could not find any evidence for endogenous analgesia in pregnant rats. Subsequent investigations have consistently shown reduced sensitivity to pain shortly before parturition in humans and in rats. Kaneko et al. reported that by the end of the first third of gestation, pregnant rats had significantly higher visceral pain thresholds compared with preganational controls and that the magnitude of this pregnancy-induced analgesia increased throughout gestation. Although this view is somewhat controversial, endorphin levels are believed to increase during preg-

nancy and labor, and an interaction between the endogenous opiate system and local anesthetics may be responsible for the increased local anesthetic sensitivity of pregnant patients. Clinically, it has been reported that intrathecal sufentanil alone for labor anesthesia can cause a segmental sensory block and even a motor block, as subjectively described by 30% of the patients in another study. It is possible that sufentanil may have a weak local anesthetic effect in pregnant patients, or, alternatively, changes at the cell membrane may reinforce a synergistic interaction between opiates and local anesthetics. A study in rats found that sufentanil analgesia can be potentiated by pretreatment with progesterone. In the present study using only isolated spinal root axons, there are no functional opiate receptors, and thus endogenous opiate receptor-mediated effects are not possible.

It has also been suggested that hormonal changes present from early in pregnancy could increase susceptibility to local anesthetics. For example, measurements of plasma and cerebrospinal fluid progesterone levels in nonpregnant, full-term pregnant, and postpartum patients showed that plasma and cerebrospinal fluid concentrations were significantly higher, and that significantly less lidocaine was needed for comparable levels of anesthesia in pregnant and postpartum patients. Datta et al. reported that in vitro exposure to bupivacaine caused a significantly more rapid depression of compound action potential amplitude in intact vagus nerves isolated from pregnant rabbits. They attributed this increased local anesthetic sensitivity to elevated progesterone levels, which were found to be five times higher in the pregnant rabbits at the time of nerve harvest. Studies examining the effect of acute exposure to progesterone on analgesia and nerve conduction in vitro in rats and in vitro on isolated rabbit vagus nerves do not support a direct effect of progesterone on neural transmission, but the possibility that chronic exposure to progesterone on neural transmission during gestation may alter nerve function cannot be excluded.

Three studies have suggested that pregnancy potentiates local anesthetic inhibition of action potential propagation in nerve fibers. In the study by Datta et al., intact vagus nerves from pregnant rabbits near full term were exposed to 350 µM bupivacaine in vitro at 22°C. The times to achieve a 50% reduction in the A-, B-, and C-fiber elevations of the compound action potential were measured. In pregnant animals these times were significantly shorter for all fiber groups, with A-fibers being the least sensitive. Thus, although it is possible that pregnancy directly increased the susceptibility of these nerve fibers to bupivacaine, it is equally possible that pregnancy changed the diffusion pathways and barriers for bupivacaine, improving access to otherwise unchanged membrane effector sites. In a subsequent study from that laboratory, intact rabbit vagus nerves were exposed to bupivacaine at concentrations from 100-1,000 µM at 22°C. Dose-response curves were constructed for the A-, B-, and C-fiber components of the compound action potential. Unlike the previous study, however, the results of this study suggested that A-fibers in vagus nerves from pregnant rabbits were more sensitive to bupivacaine than were the other fiber groups. Interpretation of these results is complicated by the fact that steady-state conditions were not achieved. Thus the apparent increased sensitivity of vagus nerves from pregnant animals may again simply reflect changes in diffusion pathways and barriers resulting in a more rapid onset of anesthetic effect. The third study examined the local anesthetic susceptibility of the median nerves in nine pregnant (third trimester) and eight nonpregnant women. Lidocaine was injected near the median nerve at the wrist, and antidromic compound action potentials were recorded during a period of 20 min from the middle finger in response to electric stimulation of the proximal median nerve. A significant time-dependent difference was noted with the sensory potential, which declined more rapidly in pregnant women; however, by 15-20 min, this difference did not appear to be significant. No significant differences were found in other measures of local anesthetic effects, including compound motor action potentials, skin temperatures, and galvanic skin potentials. As in the previous two studies, the finding of a significant temporal effect was used to support the general conclusion that pregnancy affects nerve function. However, it cannot be concluded from these studies that these effects are mediated by change in the sensitivity of the nerve cell membrane. Indeed, the time dependence of these effects supports the hypothesis that pregnancy may alter diffusion barriers or otherwise improve local anesthetic access to effector sites. Further, these three studies share a common limitation: The magnitude of conduction block cannot be measured reliably by changes in compound action potential amplitude. The amplitude of compound action potentials is determined by the number of active axons and their
degree of synchrony. Thus a reduction in compound action potential amplitude can occur as a result of changes in individual axon conduction velocities without the occurrence of conduction block. Studies purporting to measure conduction block by measuring changes in compound action potential amplitude must be interpreted with an understanding of this potentially confounding factor.

In the present study, the steady-state sensitivity of individual DR and VR axons to bupivacaine was measured in two ways: the minimum concentration necessary to produce conduction block and the concentration that increased conduction latency by 50%. By both measures, pregnancy was not associated with an increase in susceptibility to bupivacaine in either DR A-fibers, DR C-fibers, or VR fibers. Pregnancy appears to slightly decrease axonal sensitivity to bupivacaine, which is in contrast to clinical expectations. In clinical studies, the ability to measure steady-state local anesthetic effects is difficult. Most studies of local anesthetic sensitivity during pregnancy are confounded by measurements of time-dependent phenomena. Although the rapid onset and enhanced spread of spinal and epidural anesthetics during pregnancy could be the result of structural or biochemical changes at the level of the axon membrane, its ion channel, and drug receptors, the results of the present study do not support this hypothesis. The more likely explanation probably involves a combination of factors including changes in diffusion barriers and activation of endogenous analgesic systems. Reports of the local anesthetic effects of opiates and of the analgesic effects of sub-blocking concentrations of local anesthetics support the existence of a common pathway for synergistic interactions. Thus local anesthetics and opiates may be more effective during pregnancy as a result of changes at central locations (e.g., opiate receptors in the dorsal horn) while concurrent connective tissue changes improve drug access to effector sites, probably in the DR entry zone. This could explain previous clinical and laboratory observations without requiring a direct pregnancy-related change in the electrophysiologic properties of spinal root axons. In conclusion, pregnancy does not increase the susceptibility of DR or VR axons to the conduction blocking or conduction slowing effects of the local anesthetic bupivacaine. Additional studies are needed to test alternative hypotheses.

References


