Endocrine Parameters Associated with Disruption of Parturition after Bilateral Pelvic Neurectomy (43475)

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Abstract. **Bilateral lesions of the pelvic nerve (BLPN) result in dystocia, but the processes which control this effect are not fully understood. Plasma progesterone, relaxin, and luteinizing hormone (LH) concentrations were measured in blood samples taken in the morning (AM) and evening (PM) of Days 20–23 of gestation from rats with BLPN or sham neurectomy. Ten of 11 sham-operated control animals delivered their entire litters by Day 23 of gestation, but animals with BLPN did not complete parturition by Day 23 when they were sacrificed. Progesterone concentrations were greater in rats with BLPN than in sham-operated rats on Day 20 PM and Day 21 AM, but hormone concentrations declined to minimal values by Day 22 in both groups. Relaxin concentrations were greater in rats with BLPN than in sham-operated rats on Day 21 PM. Thereafter, relaxin concentrations decreased to reach minimum values on Day 23 in both groups. LH concentrations were low throughout the period of study in rats with BLPN; however, a postpartum LH surge was detected in all sham-operated animals. Data from this study indicate that the pelvic nerve does not control parturition by modulating serum relaxin and progesterone concentrations; however, these data suggest that impulses carried by the pelvic nerve influence ovarian secretion of these hormones. In addition, these data indicate that the pelvic nerve transmits stimuli from the cervix to the hypothalamus to facilitate the postpartum LH surge.** [P.S.E.B.M. 1992, Vol 201]

Although hormones and the nervous system both play important roles in control of parturition, the precise role of each system and their respective mechanisms of regulation are not known. Motor and sensory innervation to the uterine body, cervix, and vagina are supplied by the hypogastric and pelvic nerves (1–3); however, the mechanoreceptive field and types of information conveyed by each nerve are different (3). Bilateral sectioning of the pelvic nerves disrupts parturition in rats (4–6). This disruption may take the form of total inability to deliver, incomplete expulsion of a single pup, or delivery of several pups before incomplete expulsion of a pup. The effect of bilateral lesions of the pelvic nerve (BLPN) on parturition may be due partly to alteration of the hormonal milieu in the antepartum period. Louis *et al.* (6) collected blood samples at the time of sacrifice on Days 20–24 of pregnancy and reported that the prepartum fall in peripheral progesterone concentrations was delayed in rats with BLPN compared with sham-operated rats. Prostaglandin $F_{2\alpha}$ concentrations increased on Day 22 (of parturition) in sham-operated rats, but were unchanged up to Day 24 in animals with BLPN. Burden *et al.* (7) reported that estradiol concentrations increased from Day 21 to Day 22 in sham-operated rats, but were unchanged in animals with BLPN during this period. Relaxin may also have an important regulatory function in initiation and completion of parturition. Synthesized by the corpus luteum of the rat (8, 9), relaxin is first detected in serum on Day 10 of pregnancy (10). Its concentration in serum increases up to Day 21 and then decreases to low levels during the 12–24-hr period prior to parturition (10). Serum relaxin concentrations in rats with BLPN have not been measured.

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The postpartum luteinizing hormone (LH) surge occurs during the afternoon or evening of the day after parturition (11–13). Initiation of this surge may be associated with mechanical stimulation of the cervix during parturition, since the postpartum LH surge was not detected in rats with young delivered by cesarean section (14).

The objective of this study was to measure progesterone, relaxin, and LH in plasma collected twice daily during the antepartum period from chronically catheterized rats after BLPN or sham neurectomy.

Materials and Methods

Animals and Surgical Procedures. Mature female Sprague-Dawley rats (230–350 g) with two consecutive 4-day estrous cycles were bred by housing them individually with a male on the evening of proestrus. The following day was designated Day 1 of gestation, and the male was removed. Animals were housed in the Department of Comparative Medicine, East Carolina University, Greenville, NC, on a 14:10-hr light:dark cycle with free access to laboratory chow and water. On Days 8–10 of gestation, animals were anesthetized with chloral hydrate (30–35 mg/100 g body wt), and the pelvic nerves were exposed by midventral laparotomy and sectioned by the technique of Carlson and DeFeo (4). These nerves were exposed and touched, but not sectioned in sham-operated control animals. On Day 19 of gestation, animals were anesthetized with ether and a Silastic catheter (0.02 in i.d./0.037 in o.d., Dow Corning Corp., Midland, MI) was inserted into the right jugular vein. Blood samples were collected at 12-hr intervals beginning on the morning of Day 20 (0800–0900 hr; AM) and continuing through the evening of Day 23 (2000–2100 hr; PM). Blood samples (1.5 ml) were collected into heparinized syringes and centrifuged, and the plasma was stored at −20°C until assayed for hormone concentrations. Because of the large volume of each blood sample, packed cells from each bleeding were resuspended in heparinized saline and reinjected after the next blood sample was collected. At each blood-sampling period, animals were observed for evidence of initiation of delivery and the number of pups living and dead were recorded. Animals were sacrificed after the last blood sample and the uterine contents were determined.

Hormone Assays. Luteinizing hormone concentrations were measured by radioimmunoassay using reagents supplied by the Hormone Distribution Program of the National Institutes of Health. Assays were performed by the double-antibody method using the instructions provided with the reagents (LH standard was RP-1), and the sensitivity for LH was 0.7 ng/ml. Progesterone was determined by radioimmunoassay using the reagents and protocols described previously (15). Relaxin was measured in plasma samples using a heterologous double-antibody radioimmunoassay. Highly purified porcine relaxin iodinated by the method of Bolton and Hunter (16) with the modifications of McMurtry *et al.* (17) was used as the hormone tracer. Rabbit antiserum to purified rat relaxin (267-5; kindly provided by Dr. O. D. Sherwood) was described previously (18). A purified rat relaxin preparation (provided by Dr. O. D. Sherwood) was used as the assay standard. Relaxin was assayed in duplicate 5-, 10-, or 20-μl aliquots of each sample, and the assay was performed using a modification of the procedures of Sherwood and Crnekovic (18). Briefly, rat relaxin standard (7.8–4,000 pg) or unknown sample was added to 12 × 75 mm tubes, and sufficient phosphate-buffered saline (PBS) containing 1% (w/v) ovalbumin was added to bring the volume to 500 μl. Two-hundred microliters of rat relaxin antiserum (diluted 1/10,000 in 0.05 *M* EDTA-PBS and containing 1/300 male rabbit serum) were added to each tube and the assay was incubated at 4°C for 24 hr. After incubation, 100 μl of iodinated porcine relaxin (35,000–45,000 cpm diluted in PBS-1% ovalbumin) were added and incubation continued for an additional 24 hr. Next, 200 μl of sheep antiserum to rabbit IgG (diluted 1/20) were added and the assay was incubated at 4°C for 72 hr. Three milliliters of PBS were added to each tube and the tubes were centrifuged at 3,000*g* for 30 min. The supernatant was decanted and the pellet was counted in a gamma-counter. Specific binding of the iodinated porcine relaxin tracer to the rat relaxin antiserum was approximately 10%. The antiserum did not show significant cross-reactivity with insulin (0.1–100 ng), proinsulin, oxytocin, or nerve growth factor. A total of 483 ± 13.3 pg (n = 5) were recovered when 500 pg of rat relaxin were added to a pool of male rat plasma. The sensitivity of the assay was 62 pg. The linear portion of the standard curve was between 125 and 2,000 pg and was parallel to serial dilutions of plasma from late pregnant rats. Relaxin concentrations of the unknown were determined from a log-logit transformation of the standard curve. Intra- and interassay coefficients of variation were 15.5% and 14.0%, respectively.

Statistical Analysis. Hormone data were analyzed by the method of least squares using the general linear models procedures of the Statistical Analysis System (19). The overall mathematical model for all variables included effects of treatment, animals within treatment, period, and treatment × period interaction. Individual multiple comparisons were tested using the method of Scheffé (20).

Results

Pregnant rats in our colony usually initiate parturition by the evening of Day 22 of pregnancy. In this study, ten of 11 sham-operated control rats delivered their entire litters by 0900 hr on Day 23 of gestation.

In contrast, parturition was not completed by any animals with BLPN by the time of sacrifice. Nine of 15 rats with BLPN did not successfully deliver any young; however, in these animals, a partially expelled pup was usually observed at the vaginal opening. Six of 15 rats with BLPN either were not observed to attempt delivery, or they expelled several pups but did not deliver the remainder of the litter. At sacrifice, a pup was present in the cervical canal of all rats with BLPN.

Progesterone concentrations decreased in both treatment groups from Day 20 to 22 (Fig. 1). In sham-operated animals, a biphasic decrease in mean progesterone concentrations was observed; however, this pattern was caused by one animal that had a very high progesterone concentration (68.4 ng/ml) in the Day 21 PM sample. When this animal was eliminated from the analysis, a continuous decline in progesterone concentrations was observed (Fig. 1; broken line). Significant effects of treatment and the treatment × period interaction were not detected. Selected multiple comparisons indicated that progesterone concentrations on Days 20 PM and 21 AM of gestation were greater in rats with BLPN than in sham-operated animals; however, progesterone concentrations reached minimum values at the same time (Day 22 AM) in both groups.

Plasma relaxin concentrations for the period studied were significantly higher in animals with BLPN compared with sham-operated animals (Fig. 2). Relaxin concentrations in rats with BLPN were higher than those measured in sham-operated animals on Day 21 PM, but mean hormone concentrations were not different ($P \leq 0.07$) on Day 21 AM. Thereafter, relaxin concentrations decreased in both groups to reach minimum values on Day 23.

Luteinizing hormone concentrations were low (mean, 8.18 ng/ml) in BLPN animals throughout the period of the experiment (Fig. 3). One elevation in mean hormone concentration was observed in sham-operated animals on Day 23 PM. A postpartum LH surge was detected (value > 100 ng/ml) in all sham-operated animals by Day 23 PM.

Discussion

In this study, parturition was disrupted in rats after BLPN. Bilateral lesions of the pelvic nerve render rats unable to spontaneously deliver their pups (4–6), although timing of the initiation of parturition is not different from that of control rats (21). Recent evidence indicates that the afferent (sensory) component of the pelvic nerve is responsible for this physiological effect (22). Processes controlled by sensory outflow through the pelvic nerve that are necessary for successful parturition are not known with certainty; however, several hypotheses have been proposed. Higuchi *et al.* (21) observed that distension of the vagina caused contraction of abdominal muscles and the diaphragm which increased intra-abdominal pressure, presumably to facilitate fetal expulsion. Pelvic neurectomy abolished this "fetus-expulsion reflex" (21).

Another hypothesis is that changes in antepartum

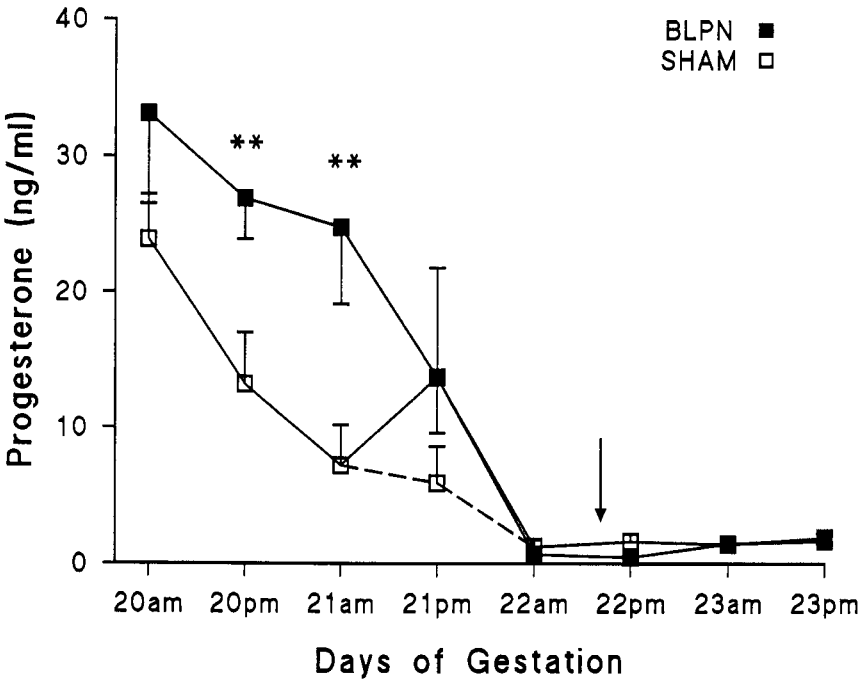

Figure 1. Plasma progesterone concentrations in rats with BLPN ($n = 9$) or sham-operated rats ($n = 8$) on Days 20–23 of gestation. $^{**}P \leq 0.05$. The broken line represents the profile after eliminating the high progesterone value of one rat from the Day 21 PM mean. The arrow indicates the mean time of initiation of parturition.

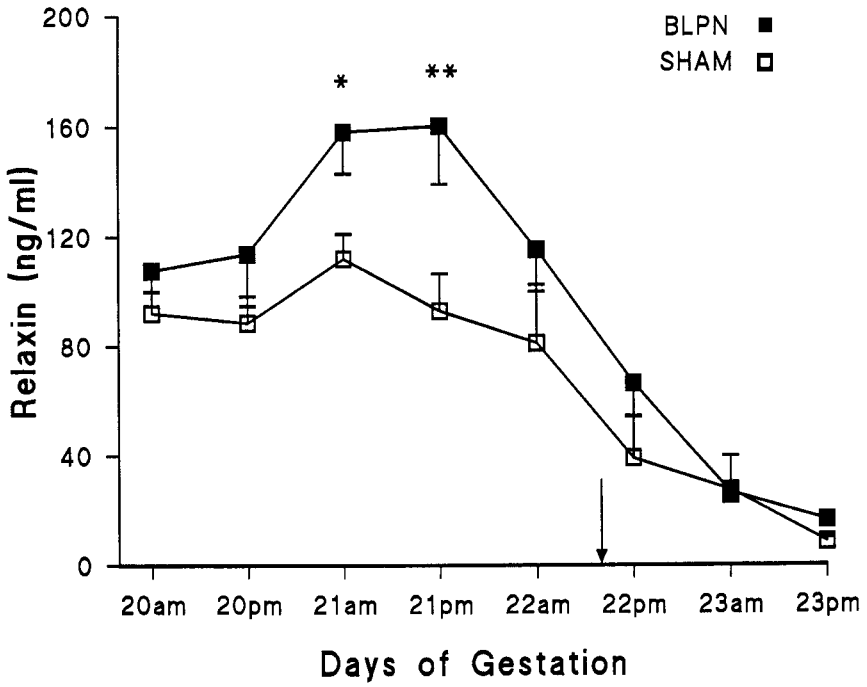

Figure 2. Plasma relaxin concentrations in rats with BLPN (n = 9) or sham-operated rats (n = 8) on Days 20–23 of gestation. $^{**}P \leq 0.05$. The arrow indicates the mean time of initiation of parturition.

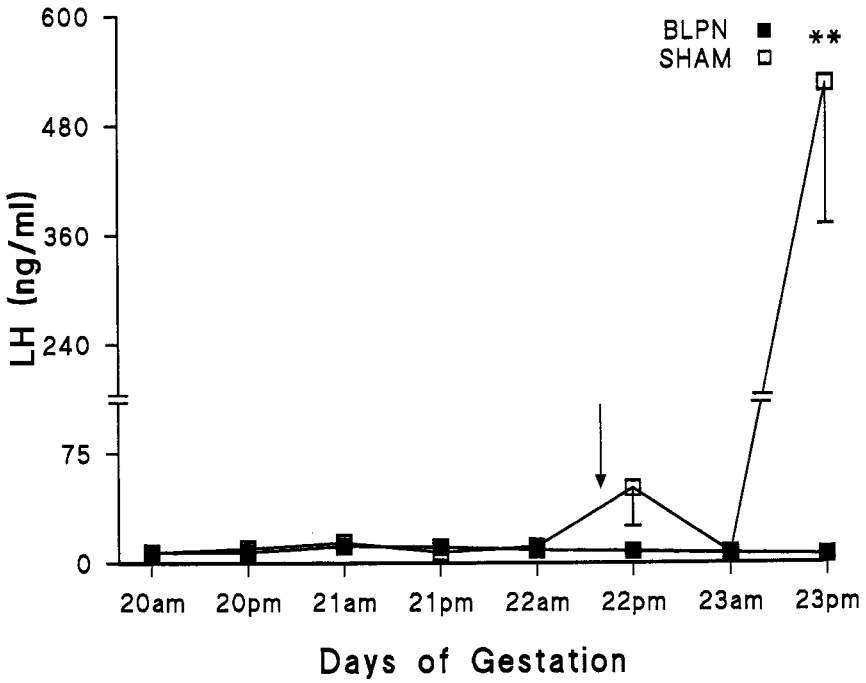

Figure 3. Plasma LH concentrations in rats with BLPN (n = 9) or sham-operated rats (n = 8) on Days 20–23 of gestation. $^{**}P \leq 0.05$. The arrow indicates the mean time of initiation of parturition.

hormone concentrations after BLPN (6, 7) may be involved. A decline in circulating progesterone after luteolysis is required for initiation of parturition in rats. Louis *et al.* (6) reported that plasma progesterone declined by Day 22 in sham-operated rats, but not in animals with BLPN, when blood samples were taken from rats before sacrifice on Days 21–24. This observation suggested that delayed luteolysis may be the mechanism for BLPN-induced disruption of parturition. In the current study, although progesterone concentrations were greater in animals with BLPN on Day 21, progesterone concentrations decreased to minimal

values by Day 22 in both groups. We believe that the sampling method used in the current study yields a more detailed profile of hormonal changes than the method used previously (6, 7). Our data indicate that the timing of the prepartum progesterone decline, and thus luteolysis, is not influenced by BLPN.

Removal of relaxin from the endocrine milieu during the latter half of pregnancy by ovariectomy and treatment with estrogen and progesterone to maintain pregnancy increased gestation length, prolonged labor and delivery (dystocia), decreased cervical extensibility, and modified uterine contractile activity compared with intact control rats (23–25). These parameters were similar to those for controls when relaxin was included in the exogenous hormone treatments. Relaxin also stimulates softening of the cervix to facilitate delivery in rats (25). We hypothesized that BLPN decreased antepartum relaxin concentrations, thereby causing a disruption of parturition. The current study indicated that relaxin concentrations were not decreased, but increased, in rats with BLPN during the antepartum period. This observation was supported by the study of Higuchi *et al.* (21), which determined that cervical distensibility was not different between rats with BLPN and sham-operated rats. Jones and Summerlee (26) reported that infusion of porcine relaxin to maintain elevated levels of serum relaxin in late pregnant rats prolonged gestation in most rats until the completion of infusion at Day 23. In those rats that delivered during the relaxin infusion, the time between the delivery of each pup was prolonged, although dystocia was not observed. Based upon this data, it is unlikely that high relaxin concentrations during the antepartum period are involved in the disruption of parturition observed in rats after BLPN.

Relaxin and progesterone are produced by the corpus luteum of pregnancy in the rat (27, 28). Although data from this study do not support the hypothesis that the pelvic nerve controls parturition by modulation of these hormones, it is apparent that impulses carried by the pelvic nerve influence ovarian function. In agreement with this conclusion is the previous observation that the prepartum rise in plasma estradiol is modified by BLPN (7).

This is the first study of plasma LH concentrations during the periparturient period in rats with BLPN. The LH surge was not detected by Day 23 PM (2000 hr) in animals with BLPN, despite efforts to deliver as evidenced by a pup retained within the cervix and/or vagina of all rats with BLPN. Fox and Smith (14) did not detect a postpartum LH surge in rats following delivery of pups by cesarean section, and they concluded that a postpartum LH surge in rats is dependent upon the cervical stimulation that normally occurs during delivery. Data from the present study indicate that afferent signals from the cervical region that regulate the postpartum LH surge are carried by the pelvic nerve. In support of this conclusion, Cunningham *et al.* (29) reported that cesarean section on Day 22 of gestation induced early ovulation in rats; however, early ovulation was blocked by transection of the pelvic or hypogastric nerves. These authors viewed a cesarean section as a positive, rather than a negative, stimulus for triggering ovulation, and concluded that cesarean section activates the pelvic and/or hypogastric nerves to trigger ovulation. In apparent contrast to the findings described above, Ying and Greep (30) reported that cesarean section on Day 22 of gestation had no effect on ovulation and the postpartum LH surge. In addition, Johnson *et al.* (31) compared ovulation times in rats after cesarean section on Day 23 with times in rats after normal delivery and found no significant difference between the two groups. The timing of the cesarean section (AM versus PM; Day 22 versus Day 23) may contribute to the varied outcomes of the studies described.

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