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Physiological Regulation of Maternal Behavior in Heifers: Roles of Genital Stimulation, Intracerebral Oxytocin Release, and Ovarian Steroids[1]

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ABSTRACT

We tested the hypotheses that 1) epidural anesthesia at parturition would block both peripheral and central release of oxytocin and eliminate the development of maternal behavior in primiparous heifers and 2) estradiol priming, genital stimulation, and appropriate neonatal stimuli would induce maternal behavior in nulliparous heifers. In experiment 1, primiparous crossbred heifers (n = 13) with cannulas in the third cerebroventricle (IIIV) were assigned randomly to receive epidural treatments of saline (SAL; n = 6) or lidocaine HCl (EPI; n = 7) at the onset of labor induced between Days 270 and 280 of gestation. Epidural anesthesia blocked ($P < 0.001$) both central and peripheral release of oxytocin and markedly reduced ($P < 0.05$) or eliminated licking behaviors during a 3-h period following parturition as compared with SAL. Following approximately 1 wk of controlled daily suckling, during which calves were permitted access only to the inguinal region of their dams (three times daily for 10 min each time), a second maternal behavior test was performed. Although licking behavior remained markedly reduced ($P < 0.001$) in the EPI compared with the SAL groups, all heifers accepted their calf at the udder. In experiments 2–4, neither estradiol priming in ovariectomized heifers nor estradiol plus progesterone in intact heifers resulted in an induction of maternal behaviors following genital stimulation and presentation of a neonate wetted with amniotic fluid. Pelvic sensory deficits apparently block oxytocin release and disturb both short-latency and long-term maternal behaviors but do not result ultimately in rejection of the calf. Combinations of hormonal, sensory, olfactory, and visual cues observed previously to induce maternal behavior in nulliparous ewes do not appear adequate for induction of maternal behavior in nulliparous heifers.

central nervous system, oxytocin, posterior pituitary, reproductive behavior

INTRODUCTION

Pregnancy and parturition serve as physiological mediators of a remarkable series of behavioral changes in female ungulates. The typical disregard for neonates exhibited by nulliparous females before and during early pregnancy is transformed into an intense interest immediately before or during fetal delivery. The onset of proceptive behaviors, including sniffing, licking, and protection of the neonate, and formation of a selective maternal bond follows [1–4]. This behavioral transformation often requires only a few minutes to complete, and the sensitive period during which the neural circuitry of the dam is capable of responding to inductive stimuli lasts no more than a few hours [5–7]. In those species studied, the induction of maternal behavior in the primiparous female is the product of a cascade of sensory and hormonal events, including exposure of the maternal brain to placental-derived estradiol, intense genitosensory stimulation, central release of oxytocin and opioid peptides, and olfactory and visual cues from the neonate [3, 4].

Although at least one study has examined the relative importance of olfaction and vision to the expression of maternal selectivity in cattle [8], the contributions of genital stimulation and central oxytocin release at parturition to the ontogeny of maternal behavior in this species are virtually unknown. A better understanding of the endocrine and sensory determinants involved in the induction of maternal care and selectivity in cattle could assist in addressing behavioral anomalies and issues related to suckling-mediated anovulation. In cattle, the maternal-offspring bond plays a major role in the neuroendocrine regulation of this type of anovulation [9]. The objectives of studies reported here were to determine the role of genital signaling at parturition on brain oxytocin release and the onset of maternal behavior in primiparous heifers and to evaluate the ability of hormonal and sensory stimuli to induce maternal behavior in nulliparous heifers.

MATERIALS AND METHODS

Experiment 1

Our hypothesis was that epidural anesthesia at parturition inhibits or delays central and peripheral oxytocin release and the establishment of maternal behavior in primiparous heifers.

Preexperimental period. Thirteen crossbred (3/4 *Bos taurus* × 1/4 *Bos indicus*) pregnant primiparous heifers in excellent body condition (BC; score of 6, where 1 = emaciated and 9 = obese) were brought in from pasture during the third trimester of pregnancy. These heifers were tamed previously for use in these studies and were accustomed to human contact. Heifers were maintained in outdoor pens, fed according to National Research Council (NRC) recommendations for late gestation [10], and acclimated to experimental conditions for 10–15 days. For acclimatization, heifers were placed in stanchions for up to 2 h daily while haltered and tied loosely to simulate experimental conditions. Between Days 267 and 270 of gestation, a third ventricle (IIIV) guide cannula was placed surgically in each heifer as described previously [11]. Silicone elastomer tubing (0.51 mm inside diameter [i.d.], 0.95 mm outside diameter [o.d.]; Silastic; Konisberg Instruments, Pasadena, CA) or polyethylene tubing (0.58 mm i.d., 0.965 mm o.d.; Intramedic and Clay Adams, Becton Dickson Co., Sparks, MD) was threaded through the IIIV guide cannula so that 5–15

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mm rested beyond the cannula tip in the IIIV. Location of the guide cannula was confirmed as described previously using lateral radiographs [11].

Experimental period. Heifers were assigned randomly to a saline control group (SAL; n = 6) or an epidural anesthesia group (EPI; n = 7). Seven to 10 days after IIIV surgery (Days 274–280 of gestation), a jugular cannula was placed in each heifer, and parturition was induced with a single injection (25 mg i.m.) of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Lutalyse; Upjohn Co., Kalamazoo, MI). Each heifer was placed indoors in an individual pen and monitored continuously until the first signs of labor, including restlessness, appearance of cervical mucus, and/or appearance of the calf's feet. All treatment and sampling procedures were performed in a relaxed setting with minimal or no restraint. Beginning at the time of injection of $PGF_{2\alpha}$, cerebrospinal fluid (CSF; 1–1.5 ml) and blood (10 ml) were collected to establish the oxytocin baseline every 6 h while heifers stood comfortably in a stanchion. Because heifers were tame and accustomed to human handling, the head was not placed in the head restraint and significant forward and lateral movement was afforded at all times. At onset of labor, each heifer was placed immediately in the stanchion and an area over the tail head was prepared for aseptic administration of epidural solutions. Physiological saline (10 ml) or lidocaine (2% lidocaine HCl; AmVet Products, Fort Collins, CO) was infused into the epidural space between the last lumbar and first caudal vertebrae. In the EPI group, lidocaine was infused ''to effect,'' defined as the presence of a limp tail, the complete absence of response to a pinch test or needle prick, the absence of the Ferguson reflex, and a flaccid vaginal vestibule [12]. In the majority of heifers, this dosage also resulted in either partial (n = 5) or complete (n = 2) paralysis of the hind legs. However, paralyzed heifers were able to remain standing as long as they were allowed to support themselves against the side panel of the stanchion. At labor onset, CSF and blood sampling was increased to every 5 min until calf delivery was complete. Samples were then collected at 1-h intervals during the 3 h after calf delivery. For both groups, the calf was delivered manually in an attempt to standardize pelvic stimuli as much as possible. A mechanical calf extractor was utilized to assist in the process as required.

During the sampling process, CSF samples were placed in 1.5-ml microcentrifuge tubes (Fisher Scientific, Pittsburgh, PA) containing 50 μl oxytocinase inhibitor (0.0372 g EDTA and 0.001 g 1,10-phenanthroline/ml of 0.05 M PBS) and were maintained in an ice bath until the end of intensive sampling. Samples were stored at −20°C until assayed for oxytocin. Blood samples were collected into 15-ml conical centrifuge tubes containing oxytocinase inhibitor (50 μl), capped, gently inverted two or three times, and maintained on ice until centrifugation at the end of the intensive sampling process. Each plasma sample was transferred to 7-ml plastic vials previously rinsed with oxytocinase inhibitor and stored at −20°C until assayed for oxytocin.

Although frequency of approaching the calf is a viable measure of maternal behavior, we did not consider it acceptable for this experiment given the compromised mobility of dams in the EPI group. Therefore, the calf was physically placed in front of any heifer in either group that did not or could not approach the calf within 5 min of delivery. Thereafter, frequencies of maternal behaviors (sniffing, licking, suckling) were recorded by a remote human observer for 1 h after calf delivery, and calves were allowed to remain with dams for 3 h with minimal human interference in the pen in which delivery occurred. During this data recording and bonding period, no human interference was allowed except under two circumstances: 1) to quickly retrieve the blood and CSF samples as described above and 2) to replace an active calf in front of its dam where the calf had moved away and the dam was unable to follow. These operations were done quietly and quickly so as to minimize any adverse effect on the behavior of the dam or calf, whereupon the observer returned immediately to his/her remote observational post. Because all dams were tame and used to human contact, we are confident that these activities had little or no effect on the experiment. Calves that did not suckle were fed with their dam's colostrum, either by bottle or stomach tube, at the end of the 3-h observation period. Because vocalization is not a prominent feature of maternal behavior in this species and did not occur consistently during the experiment, we did not include this behavior in our analysis. Beginning on the morning following parturition and continuing for 1 wk, heifers were allowed to suckle calves for 10 min three times daily in stanchions. All calves in all groups were vigorous and able to walk to and from the stanchion area without assistance and obtained all of their nourishment from their dams during the 1-wk period of controlled suckling. During each 10-min suckling bout, calves were manually placed at the udder of the cow by a human handler. No head-to-head contact, smelling, licking by the dam, or other interaction was permitted, thus avoiding any further reinforcement of the maternal/offspring bond [8, 9]. Calves were removed immediately after each suckling bout and taken to their respective pens in a location away from visual or auditory contact with dams.

At the end of 1 wk, a second maternal behavior test was performed for 15 min with each pair in an indoor pen and using procedures and observations as described above.

Experiments 2–4

Hypotheses were that genital stimulation and circulating concentrations of estradiol and progesterone interact to modulate the induction of maternal behavior in ovariectomized or intact nulliparous heifers. A series of experiments were conducted, with different hormonal regimens employed in each experiment. For each experiment, heifers served as their own control.

Experiment 2: basal estradiol in ovariectomized heifers. Five tame, nulliparous Brahman × Hereford (F_1) heifers 18–20 mo of age, weighing 368.8 ± 12.9 kg (mean ± SEM), and in excellent body condition (BC = 6) were used. Heifers were maintained as a single group in outdoor pens and were fed according to NRC recommendations for moderate growth [10]. Heifers were ovariectomized, and an estradiol implant constructed from Silastic tubing [13] and designed to maintain low basal concentrations of circulating estradiol was placed s.c. caudal to the left shoulder 6 wk before the start of the experiment [13]. Heifers were acclimated to experimental conditions daily for approximately 6 wk before start of the studies. Two days before each treatment, a jugular cannula was placed in each heifer.

Genital stimulation consisted of manual placement of a rubber soccer bladder into the vaginal vestibule with the aid of a lubricant (K-Y Jelly; Johnson and Johnson, New Brunswick, NJ). The bladder was inserted cranially against the cervix and then inflated with a manual air pump. The inflated bladder was designed to mimic vaginal distension and to cause the Ferguson reflex associated with vaginal delivery of the calf during parturition [12]. The total period of genital stimulation (10 min) involved the following: 1) rubber bladder placement and inflation lasting approximately 2.5 min, 2) inflated rubber bladder in situ for 5 min, and 3) deflation and removal lasting approximately 2.5 min.

Maternal behavior tests lasting 15 min each were performed 1 h before (control period) and immediately following the last blood sample collected after completion of stimulation. Tests utilized neonatal calves (5–10 days of age) derived from the main cow herd. Each calf was treated i.v. with a tranquilizer (0.1–0.15 ml xylazine; Miles, Shawnee Mission, KS) sufficient to maintain recumbency and to minimize movement and vocalization. Each calf was also wetted with water plus 1–1.5 L of amniotic fluid that had been obtained from a parturient cow and maintained frozen until use [3]. Observations for maternal behavior were as previously described (experiment 1).

Treatments were performed with each heifer standing calmly while loosely restrained in a stanchion. For these experiments, we used posterior pituitary release of oxytocin into the peripheral circulation as an indirect indicator of the efficacy of our procedures to induce central hormonal changes associated with genital stimulation [14, 15]. Blood samples were collected at 30, 20, 10, 5, and 0 min before stimulation and at 1-min intervals thereafter throughout the 10-min total stimulation period and until 2 min after stimulation ended. Blood was collected into chilled tubes containing an oxytocinase inhibitor as described for experiment 1. Plasma was harvested and stored at −20°C until assayed for oxytocin. An additional 10 ml of blood was collected at 30 and 5 min before genital stimulation for determination of circulating concentrations of estradiol.

Experiment 3: gestational estradiol in ovariectomized heifers. To produce circulating concentrations of estradiol that would mimic those occurring naturally during gestation, we used a modification of the protocol of Rund et al. [16]. Nulliparous heifers (n = 5) weighing 380 ± 10.2 kg were injected s.c. twice daily for 18 days with estradiol-17β (5 μg/kg body weight [BW]) dissolved in 50% ethanol (1 mg/ml). On Days 19, 20, and 21 of treatment, the dosage was increased to 10 μg/kg BW. Genital stimulation experiments, as described for experiment 2, were performed on Day 21. Blood samples were collected on Days 0, 8, 15, 19, and 21 for determination of serum estradiol. Intensive sampling for measurement of oxytocin release before, during, and after genital stimulation was as in experiment 2.

Experiment 4: gestational estradiol plus progesterone in intact heifers. Six crossbred (3/4 *B. taurus* × 1/4 *B. indicus*) sexually mature but nulliparous heifers were used in this experiment. Heifers weighed 459 ± 38.1 kg and were in excellent body condition (BC = 6). Heifers were acclimated to intensive experimental conditions for 3–4 wk as described above. During this period, each heifer was injected i.m. twice daily with estradiol-17β (5 μg/kg BW) and progesterone (0.2 mg/kg BW), both dissolved in

vegetable oil, for 20 and 19 days, respectively. Estradiol was then injected twice on Day 21 at 10 μg/kg BW. Serum concentrations of estradiol and progesterone were determined in blood samples obtained on Days 0, 8, 15, 19, and 21. Genital stimulation was performed within 24 h of the last injection, and maternal behavior tests were performed 1 h before and immediately after as described above. Intensive blood sampling was as in experiments 2 and 3.

Hormone Analyses

Intensively collected IIIV CSF (experiment 1) and plasma samples (experiments 1–4) were assayed for oxytocin concentrations using a previously validated RIA [13] with modifications, including use of an oxytocin antiserum (generously provided by Drs. G. and J. Kotwica, Academy of Agriculture and Technology and Polish Academy of Sciences, Olsztyn, Poland, respectively) as reported previously [17]. Cross-reactivity was <0.01% for other related peptides, including vasopressin, lysine vasopressin, angiotensin, vasotocin, and somastostatin. In experiment 1, sensitivity of the oxytocin RIAs for CSF and plasma averaged 2.4 and 4.2 pg/ml, respectively. Intra- and interassay coefficients of variation (CV) for CSF and plasma were 7.6 and 7.7 pg/ml, and 5.4 and 5.5 pg/ml, respectively. For experiments 2–4, assay sensitivity for plasma averaged 3.1 pg/ml, with intra- and interassay CVs of 6.9 and 6.8 pg/ml, respectively. Plasma concentrations of estradiol (experiments 2–4) and progesterone (experiment 4) were assayed by previously reported RIAs [18, 19]. Sensitivity of the estradiol assay averaged 3.9 pg/ml, with intra- and interassay CVs of 7.4% and 7.7%, respectively. Progesterone assay sensitivity was 0.02 ng/ml, with intra- and interassay CVs of 5.5 and 5.5 ng/ml, respectively.

Statistical Analyses

Plasma and CSF concentrations of oxytocin in experiment 1 were evaluated by ANOVA for a completely randomized design, with split-plot for repeated measures. Treatment, time, and all possible interactions were included in the model, with treatment effects tested using heifer within treatment as the error term. Treatment × period means were compared by the Bonferroni *t*-test after a significant *F*-value. The general linear models procedure of the Statistical Analysis System (SAS) was used to perform the analyses [20]. For experiments 2–4, an ANOVA was used to test the effects of period (before, during, and after stimulation) on circulating concentrations of oxytocin and to test day effects on circulating concentrations of progesterone and estradiol [21]. The Bonferroni *t*-test was used to contrast individual means after a significant *F*-test. The signed-rank test of Wilcoxon was employed to compare maternal behavior activities between the SAL and EPI groups (experiment 1) and before and after genital stimulation treatments (experiments 2–4) [21].

RESULTS

Experiment 1

Oxytocin release in plasma and IIIV CSF. The interval from injection of $PGF_{2\alpha}$ to onset of labor ranged from 6 to 42 h. Mean concentrations (±SEM) of oxytocin in plasma and IIIV CSF for the 360-min period before onset of labor, the 20-min period immediately before and during calf delivery, and the 120-min period following calf delivery are illustrated in Figure 1. Average baseline concentrations of oxytocin for SAL and EPI groups did not differ (plasma: 49.5 ± 6.7 pg/ml vs. 46.9 ± 5.7 pg/ml; CSF: 13.9 ± 4 pg/ml vs. 13.8 ± 2.1 pg/ml) during the baseline period and up until 15 min before calf delivery. Beginning at 10 min before calf delivery, plasma oxytocin in the SAL group began to increase, reaching a peak concentration over 2.5-fold greater ($P < 0.001$) than the EPI group at 5 min before delivery of the calf. Plasma oxytocin concentrations then began to decline, returning to baseline and values not different than the EPI group within 60 min after delivery (Fig. 1).

Between 360 min and 20 min before calf delivery, IIIV CSF oxytocin declined in the SAL group and was lower ($P < 0.05$) relative to the EPI group at −20 min. Thereafter, IIIV CSF concentrations of oxytocin began to increase linearly to values two- to threefold greater than those of the EPI group at 60 min after calf delivery and remained elevated at 120 min ($P < 0.05$–0.002; Fig. 1). Conversely, IIIV CSF oxytocin in the EPI group declined from −20 min to the time of calf delivery and remained unchanged throughout the remainder of the sampling period. One EPI heifer had to be eliminated from the analyses because of

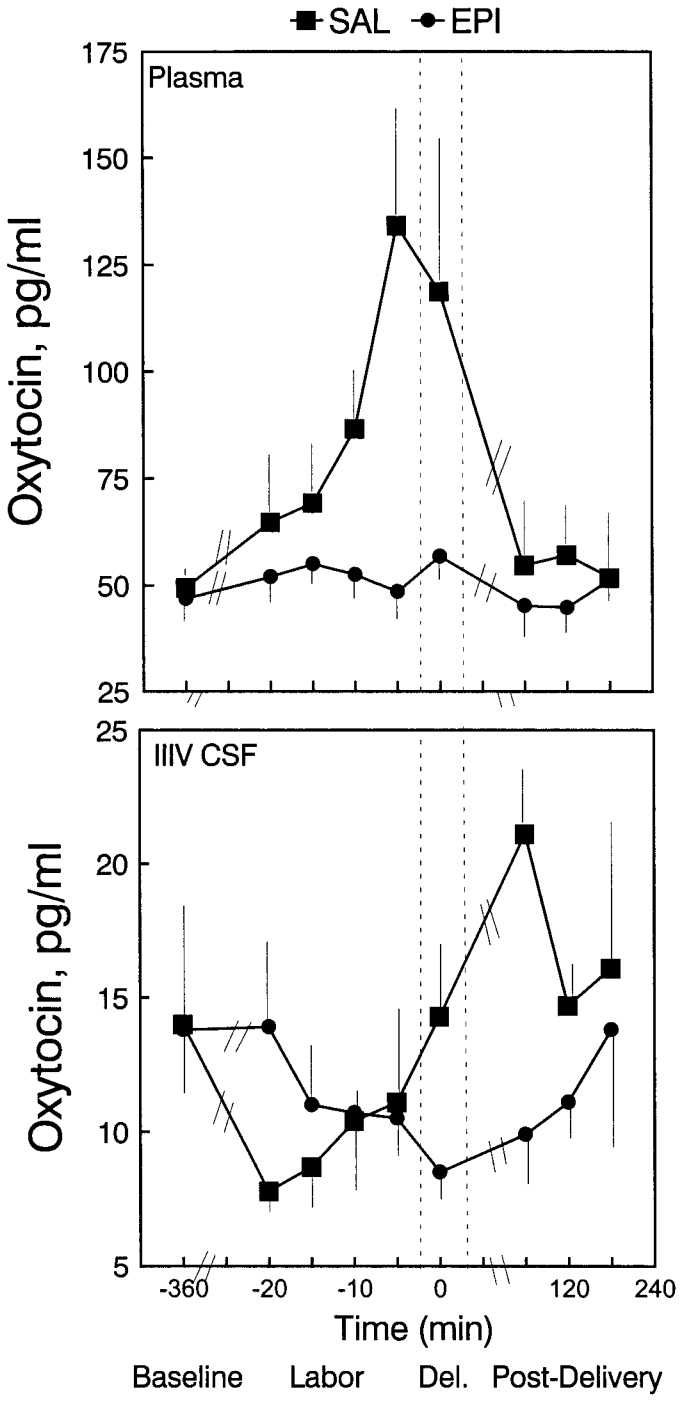

FIG. 1. Plasma (top panel) and IIIV CSF (bottom panel) concentrations of oxytocin in SAL (saline) and EPI (epidural anesthesia) heifers before onset of labor (baseline), during labor and delivery (Del), and for 3 h after delivery (experiment 1). Plasma concentrations of oxytocin were greater ($P < 0.001$) in the SAL than in the EPI group at 10 and 5 min before and during calf delivery. Concentrations of oxytocin in IIIV CSF were greater in the SAL group than in the EPI group during calf delivery ($P < 0.05$) and at 60 and 120 min after delivery ($P < 0.002$).

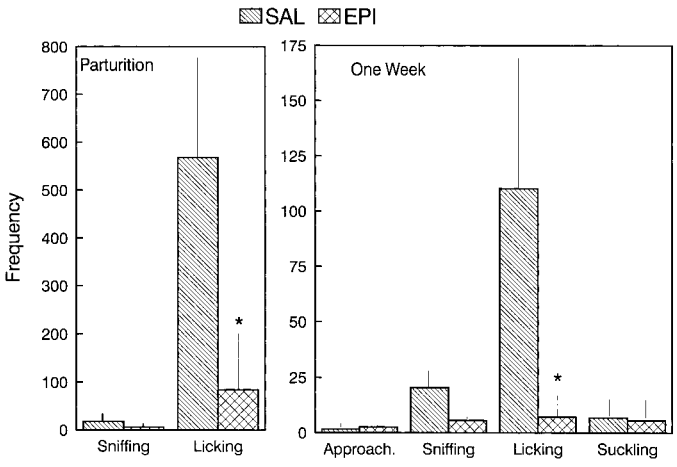

FIG. 2. Frequencies of sniffing and licking during the 1-h period after parturition (left panel) and during a maternal behavior test at 1 wk postpartum (right panel) in saline (SAL)- and lidocaine-treated (EPI) heifers (experiment 1). An asterisk denotes a significant difference between groups ($P < 0.05$).

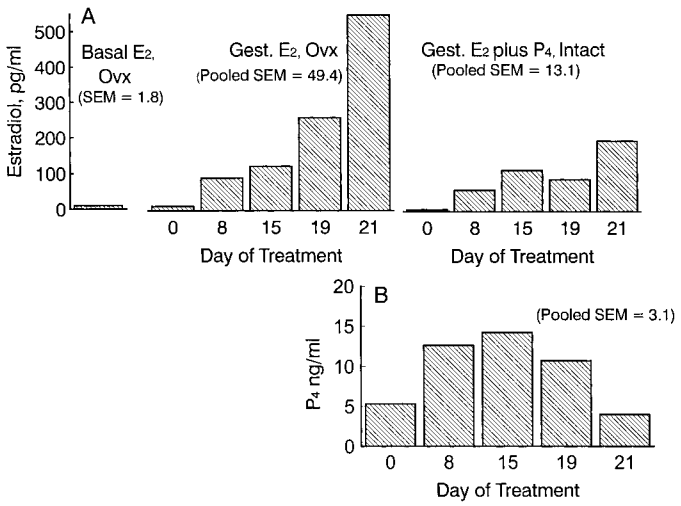

FIG. 3. Plasma concentrations of circulating estradiol (E_2) and progesterone (P_4) in nulliparous ovariectomized (Ovx) or intact heifers treated with basal or gestational levels of E_2 or E_2 plus P_4 in experiments 2–4. Variation around the means are presented as pooled SEMs for each experiment.

failure of the epidural and concomitant increases in peripheral and central oxytocin.

Maternal behavior. Maternal behavior activity is summarized in Figure 2. Calves were presented to all heifers failing to approach the calf independently based on rationale explained previously. During the first hour after calving, the frequency of sniffing the calf did not differ between SAL and EPI groups. However, a six- to sevenfold reduction ($P < 0.05$) in licking activity was observed in the EPI compared with the SAL control group (Fig. 2). No suckling activity was recorded in either group during the 3-h observation period after calving, probably because of the compromised musculoskeletal dynamics of calves delivered manually through a limited pelvic space in these primiparous heifers. However, all calves were fed colostrum from their dams using a stomach tube to ensure acquisition of passive immunity.

As described previously, pairs were maintained separately for the following week to eliminate any physical, visual, or vocal contact while separated, and no head-to-head contact, sniffing, or licking was allowed during the three daily suckling periods. During the second maternal behavior test at the end of this period, no differences were observed for frequencies of approaching or sniffing. All but two calves (one in each group) were readily accepted at the udder by dams in both groups during the 15-min test, and the remaining two were allowed to suckle within 30–60 min. However, as during the first hour after parturition, the SAL group exhibited a markedly greater ($P < 0.05$) frequency of licking behaviors than did the EPI group (Fig. 2).

Experiments 2–4

Figure 3 shows mean concentrations of circulating estradiol in ovariectomized heifers treated with low (basal estradiol, experiment 2) or high (gestational estradiol, experiment 3) levels of estradiol and circulating concentrations of estradiol and progesterone in intact heifers treated with high levels of both steroids (gestational estradiol plus progesterone; experiment 4). Concentrations of these steroids generally followed patterns expected for each respective treatment. However, oil-based treatments used in experiment 4 produced lower maximal concentrations of estradiol on Day 21 of treatment than those attained in experiment 3. In these experiments, we used peripheral oxytocin release as an indirect marker for intracerebroventricular release, and mean concentrations of oxytocin in plasma after genital stimulation for each experiment are shown (Fig. 4). Comparison of basal concentrations of oxytocin across experiments revealed that ovariectomized heifers with gestational levels of circulating estradiol had the highest oxytocin baseline, and intact heifers treated with both steroids had the next highest baseline. A small increase ($P < 0.05$) in plasma oxytocin was observed after genital stimulation in heifers having basal concentrations of estradiol (experi-

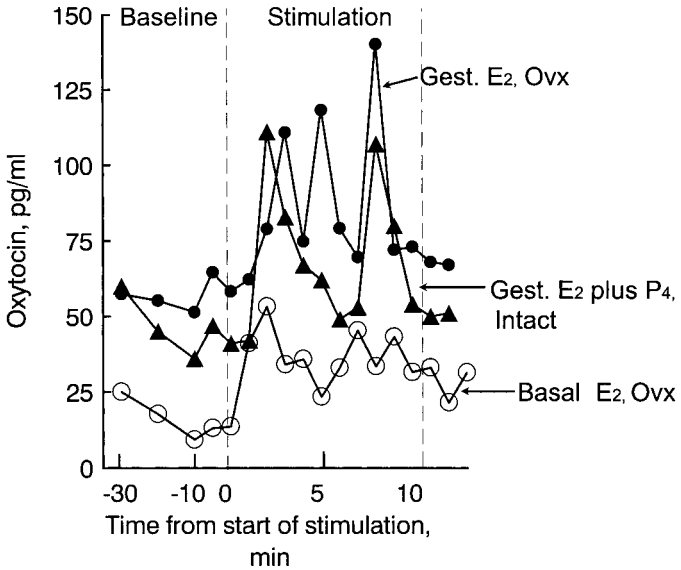

FIG. 4. Plasma concentrations of oxytocin in nulliparous heifers in experiments 2–4 before, during, and after genital stimulation. Treatments: basal estradiol, ovariectomized (Basal E2, Ovx); gestational E_2, ovariectomized (Gest. E_2, Ovx); and gestational E_2 plus progesterone, intact (Gest. E_2 plus P_4, Intact). Both basal and poststimulation concentrations of oxytocin were greater ($P < 0.05$) in experiments 3 and 4 than in experiment 2. Plasma oxytocin increased ($P < 0.01$) after genital stimulation in all three experiments compared with prestimulation values. Pooled SEMs for oxytocin means in Basal E_2, Gest. E_2, and Gest. E_2 plus P_4 groups were 2.73, 18.6, and 14.8, respectively.

ment 2), with a 2.0- to 2.5-fold increase ($P < 0.01$) observed after genital stimulation in both experiments 3 and 4. However, genital stimulation failed to induce measurable changes in the frequency of proceptive behaviors in the three experiments compared with pretreatment activities. Mean (±SEM) frequencies of approaching, sniffing, and licking of a neonate before and after genital stimulation in all experiments were 2.5 ± 0.6, 1.8 ± 0.6, and 0.6 ± 0.4, respectively.

DISCUSSION

Experiment 1

Objectives of experiments reported herein were to determine whether intracerebral oxytocin release occurs in cattle during parturition, whether epidural anesthesia inhibits this release (subsequently impairing maternal responses to the neonate), and whether treatments previously reported to be successful for inducing maternal behavior in sheep would be successful in heifers. Results confirm observations in sheep; oxytocin is released within the brain at parturition, and this release can be measured in CSF [22, 23]. Both natural [15] and artificial [14] stimulation of genitalia, including uterus, cervix, and vagina, have been reported to cause central oxytocin release, occurring coincident with the neurohypophyseal release of oxytocin into the peripheral circulation. Our observations in the current study that peripheral concentrations of oxytocin increased and peaked about 10 min earlier than IIIV CSF concentrations are in contrast to previous reports in sheep [14]. In sheep, synchronous peaks of peripheral and central oxytocin in response to vaginocervical stimulation were observed. This difference in synchrony could be related to the precise timing or frequency of sampling in the two studies and the fact that our brain cannulas were in the IIIV instead of the lateral ventricles as in the sheep study. Oxytocin in IIIV CSF remained elevated for at least 120 min after parturition, which is longer than reported after vaginocervical stimulation [14] but in agreement with times reported after parturition in sheep [24]. The source(s) of oxytocin release within the brain has been controversial. Oxytocin-containing neurons and receptors have been identified in virtually every part of the brain that has been implicated in the control of maternal behavior, especially hypothalmic and other limbic areas, including the paraventricular nucleus, medial preoptic area (MPOA), and the bed nucleus of the stria terminalis [24]. Immunoreactivity increases in these areas during parturition [24], and oxytocin levels increase in the CSF [22]. Increased oxytocin receptor binding has also been reported in the amygdala, MPOA, ventral tegmental area, and substantia nigra of the rat during late gestation [25].

The significant difference in CSF oxytocin observed between the EPI and SAL groups at 20 min before labor cannot be explained at this time but did not appear to influence the outcome of the study in any way. Epidural anesthesia clearly blocked both neurohypophyseal and central release of oxytocin in primiparous heifers in this study, and reduced levels of oxytocin were accompanied by a marked suppression of licking behavior during the first hour after parturition. In a similar study that did not include measurements of oxytocin release, peridural anesthesia profoundly delayed but did not prevent onset of maternal behavior in primiparous ewes and had little effect in multiparous ewes [26]. In that study, hindlimb paralysis was used as a marker for successful epidural anesthesia but was found retrospectively to be unrelated to the presence or absence of maternal behavior. Therefore, hindlimb paralysis was not used as a marker for successful epidural anesthesia in the current study, although partial (n = 5) or total (n = 2) paralysis did occur. Instead, absence of the Ferguson reflex, a flaccid vaginal vestibule following hand insertion into the vagina, and failure to respond to a pin prick were used to confirm successful regional anesthesia. In all heifers in which these criteria were met, both central and peripheral release of oxytocin were completely blocked. We observed no qualitative differences in maternal behaviors of EPI heifers that were totally vs. partially paralyzed.

Although licking behavior was either absent or limited in the majority of EPI heifers immediately after calving, visual and olfactory inputs occurring at this time were obviously adequate for the heifers to exhibit profound maternal behavior when tested 1 wk later. We have reported previously that cattle can use either vision or olfaction to effectively identify their own offspring after a selective maternal bond has been formed [8], but given the lack of interest by the EPI group in their calves at parturition in this study, we were somewhat surprised at the clear presence of proceptive behaviors when tested after 1 wk. It could be argued that the three daily suckling bouts before the final test facilitated the establishment of maternal behavior. However, each calf was brought to its dam's udder and allowed to suckle for 10 min during each bout without allowing head-to-head interaction, smelling with close oronasal contact, or licking by the dam. Because the sensitive period during which ruminant species can develop a selective maternal bond lasts for only a few hours [27], it would not be expected that proceptive maternal behaviors would be present after 1 wk unless a selective (or at least permissive) bond had already been formed. Unfortunately, we did not test whether the heifers would accept any calf or only their own in this experiment. Results of the study are similar to those observed in primiparous ewes, where epidural anesthesia also failed to alter the ultimate establishment of maternal behavior, although short latency maternal behavior was markedly disturbed [26].

Experiments 2–4

Estradiol priming is an important factor in the ability of genital stimulation or centrally infused oxytocin to induce maternal behavior in sheep [3]. The effects of central infusion of oxytocin on maternal behavior in estradiol-primed nonparturient ewes were essentially indistinguishable from those obtained with vaginocervical stimulation [28]. However, in the current studies, we were unable to find a combination of estradiol/progesterone priming, vaginocervical stimulation, and neonatal cues sufficient to produce maternal behavior in the nulliparous heifer. Circulating concentrations of total estrogens (estradiol, estrone, and estriol) increase throughout early to midgestation in cattle to between 500 and 700 pg/ml, exhibit a doubling during the last 5–10 days of pregnancy to between 1200 and 1500 pg/ml, and drop precipitously to baseline at parturition [29, 30]. Progesterone increases slowly until around Day 250 of gestation and then exhibits a precipitous drop at parturition. Mean concentrations of estradiol, the primary biologically active estrogen, and progesterone in beef cows were 217 ± 26.7 pg/ml and 3.5 ± 0.2 ng/ml, respectively, 9 ± 2 days before parturition [16]. Based on these reports, we attempted in experiments 3 and 4 to produce concentrations of estradiol on Day 21 that would be as high or higher than those reported at 9 days before parturition [16]. Our treatment regimen for experiment 3 yielded mean concentrations of estradiol of 275 ± 21 pg/ml and 525 ± 61 pg/ml, respectively, on Days 19 and 21 of treatment immediately before genital stimulation. These values are clearly within the physiological range of those encountered at calving, although the induction of maternal behaviors was not achieved. In ex-

periment 4, maximal levels of estradiol were approximately 200 pg/ml, with a declining progesterone titer just before genital stimulation, similar to that observed just before parturition [29, 30]. Again, these treatments combined with other factors did not induce maternal behavior. However, approximately 50% of ewes at estrus can be induced to exhibit maternal behavior [27]. This finding suggests that gestational levels of estradiol are not essential to sensitize the brain to oxytocin; circulating concentrations of estradiol in sheep during estrus are usually 10–15 pg/ml [31], at least 10-fold lower than concentrations observed during gestation or produced by the experimental protocols described herein.

The steroidal hormone milieu produced in experiments 3 and 4 resulted in basal concentrations and stimulated release of plasma oxytocin that were similar to those present during fetal delivery in pregnant heifers (experiment 1). Because peripheral and central release of oxytocin are highly correlated after genital stimulation, it appears that factors other than central oxytocin release were responsible for failure of these treatments to induce maternal behavior. The effect of genital stimulation on oxytocin release is potentiated by morphine in sheep, and neither oxytocin infusion nor genital stimulation produced maternal behavior of a quality equal to that observed with normal parturition unless an opiate agonist was given simultaneously [32]. Conversely, opioid antagonists tend to reduce the release of oxytocin in response to genital stimulation in ewes, but because no increase in maternal behavior was observed after genital stimulation in our experiments, the effects of an opioid antagonist could not be properly evaluated. All of the foregoing behavioral responses to genital stimulation and central oxytocin are dependent upon the influence of estradiol and its effects on the upregulation of mRNA for oxytocin and oxytocin receptors [24]. The specifics of these relationships, particularly in cattle, remain unclear. However, although the natural pattern of central oxytocin release and the development of maternal behavior at parturition in heifers are similar to those reported in the primiparous ewe, combinations of genital and hormonal stimuli shown previously to be effective for artificial induction of proceptive behaviors in sheep were generally unrewarding in the nulliparous heifer. Therefore, it can be assumed that the conditions necessary for creating changes in chemical events within the brain required for induction of maternal behavior in this species were not met in these experiments.

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