Roles of Leukotrienes in Bovine Corpus Luteum Regression: An *in Vivo* Microdialysis Study (44158)

ROBERT M. BLAIR,* RAY SAATMAN,† SHUENN S. LIOU,* JOANNE E. FORTUNE,† AND WILLIAM HANSEL*,[1]

Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, Louisiana 70808; and Department and Section of Physiology,† College of Veterinary Medicine, Cornell University, Ithaca, New York 14853*

Abstract. **A microdialysis system (MDS) was surgically implanted into the corpora lutea (CL) of 12 normally cycling Holstein heifers. Heifers were either allowed to undergo spontaneous luteolysis (Spontaneous, $n = 6$) or received an intramuscular injection of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on Day 12 of the estrous cycle (Induced, $n = 6$). The MDS was implanted on Day 11 in the Induced heifers and on Day 17 in Spontaneous heifers. CL were perfused with Ringer's solution at a flow rate of 3 ml/hr beginning immediately after surgery. Dialysate samples were collected hourly for 3–4 days. Samples were assayed for progesterone (P_4), oxytocin (OT), PGF, and leukotrienes B (LTB) and C (LTC). Dialysate OT was undetected in all but one Spontaneous and one Induced heifer. Lipoxygenase products of arachidonic acid (AA) metabolism (LTB and LTC) in the dialysate were found to be closely associated with luteal regression. In Spontaneous heifers, the mean interval from the first hormone peak to the onset of P_4 decline was similar for PGF, LTB, and LTC, with the first peak occurring at 12.8 ± 8.1, 22.0 ± 6.1, and 11.0 ± 8.9 hr before the onset of P_4 decline, respectively. The peak LTC value was greater ($P < 0.05$) than peak LTB or PGF. The 12-hr sampling interval with the highest LTC peak frequency was highly correlated ($r = 1.0$; $P < 0.01$) with the onset of P_4 decline, but the highest LTB and PGF peak frequencies were not associated with the onset of P_4 decline. Indeed, the mean numbers of PGF and LTB hormone peaks were higher ($P < 0.05$) after the onset of P_4 decline than before. Administration of $PGF_{2\alpha}$ on Day 12 of the estrous cycle stimulated a decline in P_4 secretion and an increase in the secretion of PGF, LTB, and LTC from the CL. In Induced animals, the peak level of PGF was greater ($P < 0.05$) than peak LTB. These results suggest that the AA metabolites LTB and, especially, LTC play important roles during normal regression of the bovine CL.** [P.S.E.B.M. 1997 Vol 216]

Luteolysis is necessary to facilitate estrous cyclicity as a means of providing multiple chances for the establishment of a successful pregnancy. Extensive knowledge concerning luteolysis has accumulated (1–7); however, the precise cellular mechanisms responsible for corpora lutea (CL) regression remain unclear. It is widely believed that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) released from the uterus under the influence of oxytocin (OT) released from the CL is responsible for luteolysis in ruminants (8–13). Luteolysis in ruminants can be induced prematurely by intrauterine, intravenous, or intramuscular administration of exogenous $PGF_{2\alpha}$ (8, 14–16). However, addition of $PGF_{2\alpha}$ to mixed luteal cell cultures and cultures of small luteal cells stimulates progesterone (P_4) production (1). Addition of $PGF_{2\alpha}$ to large-luteal cell preparations has no effect on basal P_4 production but inhibits the limited P_4 production stimulated by the addition of large amounts of luteinizing hormone (1). Girsh *et al.* (17) recently reported similar results for large luteal-like cells co-cultured with endothelial cells and stimulated to produce P_4. Recently, it has been shown that the administration of exogenous $PGF_{2\alpha}$ directly into the CL by an *in vivo* microdialysis system (MDS) re-

[1] To whom requests for reprints should be addressed at: Pennington Biomedical Research Center, Louisiana State University, 6400 Perkins Road, Baton Rouge, LA 70808.

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sults in the stimulation of P_4 production, suggesting that $PGF_{2\alpha}$ can also be luteotrophic *in vivo* (18).

The role of luteal OT in regression of the bovine CL is unclear (5), despite the fact that an inhibitory effect of exogenous OT on CL development was first reported in 1959 (19). It is evident that $PGF_{2\alpha}$ induces the secretion of OT from bovine large luteal cells when administered early (Days 6–12) in the estrous cycle (20–24). However, plasma OT levels appear to be at their nadir near the time of luteolysis, and OT and its mRNA are absent or extremely low in luteal tissue at this time (25, 26).

Currently, there is increasing evidence suggesting that arachidonic acid (AA) and its 5-lipoxygenase (5-LO) metabolites may be key factors in regression of the bovine CL. Hansel *et al.* (1) indicated that AA exerts many of the same effects as $PGF_{2\alpha}$ on bovine luteal cells and is able to stimulate luteolysis in the cow even in the absence of connections between the CL-bearing ovary and the ipsilateral uterine horn (27, 28). The intrauterine administration of nordihydroguaiaretic acid (NDGA), a lipoxygenase inhibitor, delayed luteolysis and extended the interestrous intervals of both cows (29) and sheep (30, 31). Administration of $PGF_{2\alpha}$ induced an increase in luteal content of leukotriene B_4 (LTB_4) in ewes (32) and increased LTB_4 production from late diestrous equine luteal cells (33). Taken together, these data implicate AA and its 5-LO metabolites as potential mediators of luteolysis in the cow.

However, there are no reports of *in vivo* measurements of the 5-LO metabolites of AA or of their relationship to luteal regression. The objective of the present study was to examine the local secretion of P_4, AA metabolites, and OT from the bovine CL during spontaneous and induced luteolysis. These two groups of animals were included to (i) characterize the secretion of these hormones during spontaneous CL regression and (ii) determine if these hormones are responsive to exogenous $PGF_{2\alpha}$ in a manner similar to normal regression.

Materials and Methods

Animals. In a preliminary trial, two normally cycling Holstein heifers (one "Spontaneous" and one "Induced") were implanted with an MDS and treated as described below in order to determine an appropriate sampling protocol and practice the *in vivo* microdialysis technique. Dialysate samples from these two heifers were assayed for P_4, PGF, and LTB.

Following the preliminary trial, 10 normally cycling Holstein heifers (approximately 2 years of age) were randomly assigned to one of two treatment groups. Heifers were either allowed to undergo spontaneous luteolysis (Spontaneous, $n = 5$) or received an intramuscular injection of $PGF_{2\alpha}$ (25 mg of Lutalyse®) on Day 12 of the estrous cycle to induce luteolysis (Induced, $n = 5$). An MDS was implanted on Day 11 in Induced heifers and on Day 17 in Spontaneous heifers. Following MDS implantation, heifers were housed in a climate controlled room (22°C) in individual stalls for the duration of the sampling period. All heifers received 20 kg of corn silage/day and were allowed *ad libitum* access to hay and water.

Installation of the Microdialysis System. The MDS was designed according to Jarry *et al.* (23). Dialysis tubing (Fresenius SPS 960, Frankfurt, Germany; MW cutoff = 1,000,000 Daltons; o.d. = 500 μm; i.d. = 340 μm) was cut to a length of 8–10 mm (active area ~5 mm) and connected to silastic tubing (Dow Corning, Midland, MI; o.d. = 0.635 mm; i.d. = 0.305 mm) with 26-gauge, 3/8-in. needle connectors and epoxy glue. The ovary containing the CL was exteriorized by flank laparotomy. Local anesthesia was induced by administration (sc and im) of 2% Procaine. An incision in the flank was made just caudal to the center of the hip bone and the ovary exteriorized. The MDS was threaded through the tissue such that only the dialysis tubing was embedded within the CL. The MDS was glued to the CL at the entrance and exit points to secure it in place, and the ovary was replaced into the peritoneal cavity. The silastic tubing was exteriorized through a small puncture in the paralumbar fossa and connected to teflon tubing (Cole Parmer, Niles, IL) on both ends. A bandage was placed over the exit site to further secure the MDS and the flank incision closed. For perfusion, one end of the tubing was connected to a syringe pump (KDScientific Model 100, Cole Parmer), while the other was connected to a fraction collector (Model 2110; Bio-Rad, Hercules, CA). Recovery rates of substances across the MDS were determined as previously described (23). In the present study, recovery rates were approximately 1% for P_4; 0.1% for OT, LTB_4, and leukotriene C_4 (LTC_4); and 0.3% for $PGF_{2\alpha}$.

Dialysate Sampling. The MDS was calibrated for a perfusion rate of 3 ml/hr with Ringer's solution, and perfusion of the CL was started immediately after surgery. However, animals were allowed approximately 12 hr to recover from surgery prior to the initiation of sample collection. For Spontaneous heifers, sample collection was initiated at 12:00 AM on Day 18 of the estrous cycle and carried out at hourly intervals through 9:00 AM on Day 21. For Induced heifers, dialysate collections were started at 12:00 AM on Day 12 of the estrous cycle, and samples were collected hourly for 72 hr following $PGF_{2\alpha}$ administration (09:00 AM on Day 12 of the estrous cycle). Dialysate samples to be analyzed for PGF and LTs were acidified (pH = 4–5) to prevent degradation. All samples were frozen (–20°C) immediately after the 1-hr collection period.

Blood Sampling. Jugular blood samples were collected twice daily at 0600 and 1800 hr. Blood samples were immediately centrifuged (1500*g* for 15 min at 4°C) and plasma harvested. Plasma was stored at –20°C until assayed for P_4 and OT.

Enzyme Immunoassays. Dialysate samples were analyzed for $PGF_{2\alpha}$, LTB_4, and LTC_4 using commercially available enzyme immunoassay (EIA) kits (Cayman Chemi-

cal Co., Ann Arbor, MI) according to the manufacturer's instructions. Assay sensitivities were 9.8, 2.2, and 9.4 pg/ml for $PGF_{2\alpha}$, LTB_4, and LTC_4, respectively. Intra- and interassay CVs were less than 10% for all EIA kits. Cross-reactivity of the EIA kits were as follows: $PGF_{2\alpha}$ cross-reacted 100% with $PGF_{1\alpha}$ and $PGF_{3\alpha}$, 7% with PGD_2, and less than 2% with all others tested; LTB_4 cross-reacted 100% with LTB_5, 39% with 6-*trans*-LTB_4, and less than 1% with all others tested; LTC_4 cross-reacted 100% with LTC_5, 47% with LTD_4 and LTD_5, 28% with *N*-acetyl-LTE_4, 7% with LTE_5, and less than 2% for all others tested. Therefore, the hormones assayed will be referred to as PGF, LTB, and LTC.

Progesterone Radioimmunoassay. Dialysate and plasma samples were assayed for P_4 using a commercially available radioimmunoassay (RIA) kit (Diagnostic Systems Laboratories, Webster, TX). Detection limit of the RIA was 0.12 ng/ml. The intra- and interassay CVs were 5.3% and 9.3%, respectively.

Oxytocin Radioimmunoassay. Oxytocin levels were measured in plasma and dialysate samples by RIA as previously described (22, 34). Dr. Dieter Schams (Institute of Physiology, Technical University of Munich, Germany) generously supplied the OT antibody. The assay sensitivity of the OT RIA was 0.25 pg/ml, and intra- and interassay CVs were 11.6% and 13.2%, respectively.

Statistical Analysis. Hormone levels and characteristics of hormone profiles were analyzed using the General Linear Models (GLM) procedure of SAS (35) with treatment (Spontaneous versus Induced) and hormone included in the model statement. To examine differences in hormone peaks among LTB, LTC, and PGF, an overall mean and standard deviation for each hormone within each animal was calculated. Peaks were defined as hormone values greater than the mean plus one standard deviation. Differences in the number of peaks before and after the onset of P_4 decline for each hormone were analyzed using the GLM procedure of SAS with treatment and the number of peaks in each period included in the model statement. To determine differences in peak frequencies (number of peaks/hr) over time, the entire sampling period was divided into individual 12-hr periods. Peak frequencies for LTB, LTC, and PGF were analyzed using the GLM procedure of SAS with treatment and hormone included in the model statement. The relationships among (i) the 12-hr period with the highest peak frequency, (ii) the 12-hr period containing the absolute peak hormone value for each hormone, and (iii) the 12-hr period during which the onset of P_4 decline occurred were analyzed using Pearson's correlation. Analyses were run both with and without the data from the two preliminary heifers. Since inclusion of the data from the two preliminary heifers did not alter the statistical analyses, the final analyses were conducted with the preliminary data included. Differences with a $P < 0.05$ were considered statistically significant.

Results

All heifers receiving exogenous $PGF_{2\alpha}$ and four of six control heifers exhibited luteolysis as defined by plasma P_4 values less than 1.0 ng/ml during the collection period. Luteolysis occurred at 20.5 ± 1.32 and 13.6 ± 0.42 days post-estrus for Spontaneous and Induced heifers, respectively. Profiles of plasma P_4 and dialysate P_4 for individual heifers were similar for all animals (Fig. 1).

In all animals exhibiting luteolysis, regardless of treatment, PGF, LTB, and LTC were evident in the dialysate. Mean dialysate concentrations of PGF, LTB, and LTC are shown along with mean P_4 concentrations in Figures 2 and 3. In general, these hormones were present in the dialysate samples with increasing frequency and at higher levels as P_4 declined during luteolysis. This was especially evident in the Induced heifers where $PGF_{2\alpha}$ administration consistently stimulated the release of endogenous LTB, LTC, and PGF from the CL.

Dialysate OT was undetected in all but one Spontaneous and one Induced heifer. In the one Induced heifer, $PGF_{2\alpha}$ administration stimulated a sharp rise in dialysate OT, which then remained detectable for an extended period of time. However, the Spontaneous heifer that exhibited OT

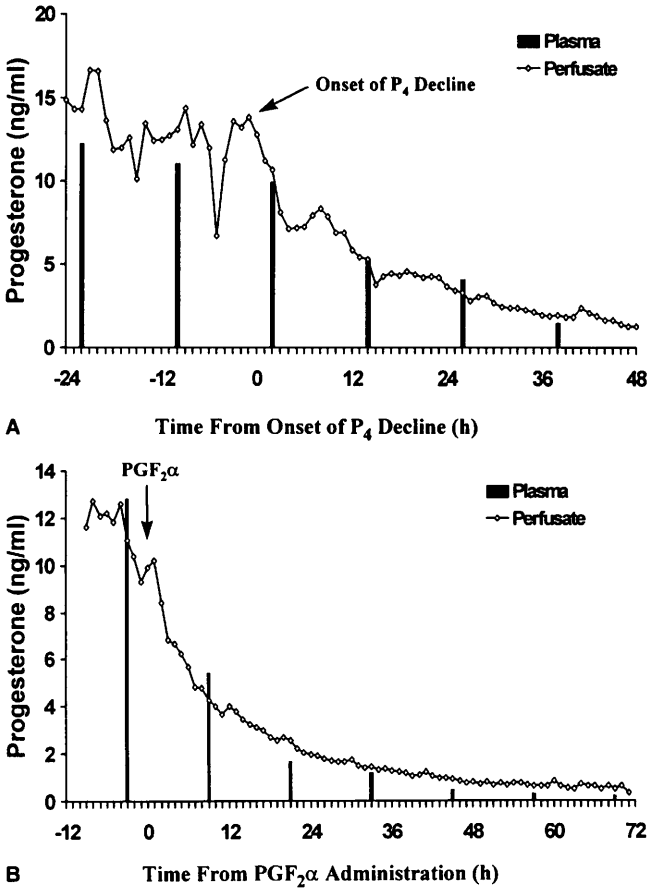

Figure 1. Mean P_4 concentrations in the dialysate (line) and the peripheral plasma (bars) of (A) Spontaneous ($n = 4$) and (B) Induced ($n = 6$) heifers. $PGF_{2\alpha}$ was administered at 0900 hr on day 12 of the estrous cycle in the Induced heifers.

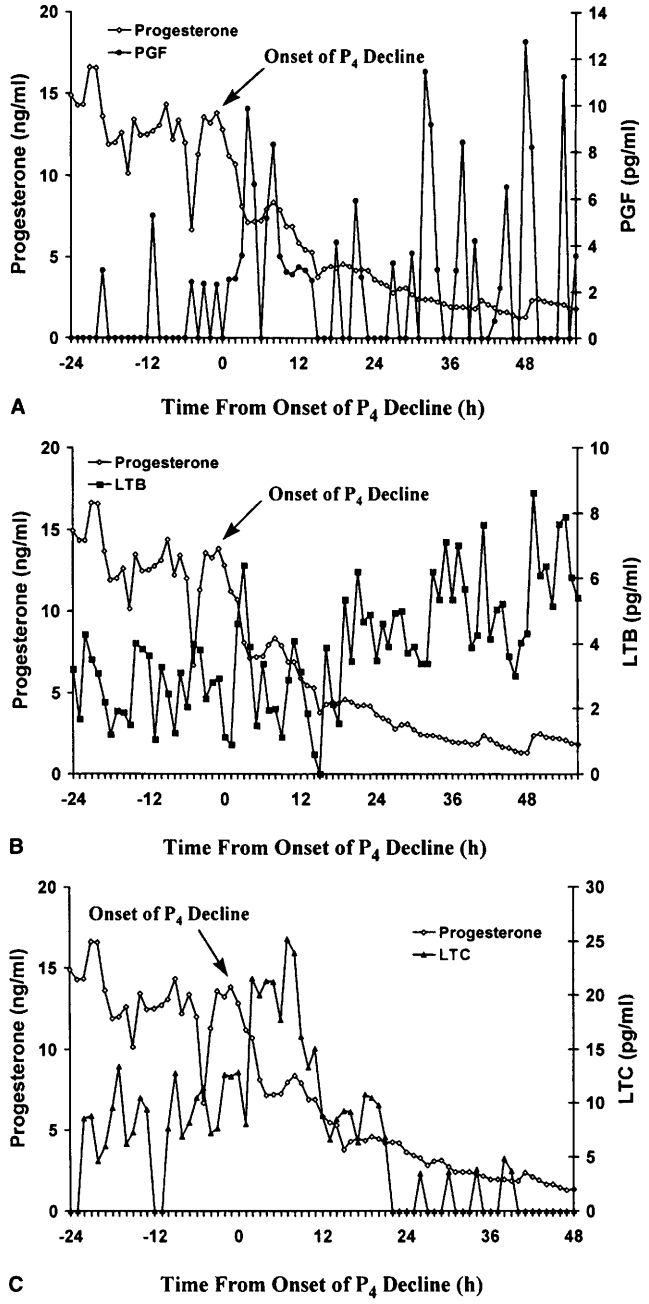

Figure 2. Mean dialysate hormone profiles of (A) PGF, (B) LTB, and (C) LTC along with the mean P_4 dialysate profile from Spontaneous heifers. Note that PGF, though present early in the sampling period became more frequent and at higher levels as P_4 declined, whereas LTB was consistently present over the duration of the sampling period and LTC was primarily secreted around the onset of P_4 decline.

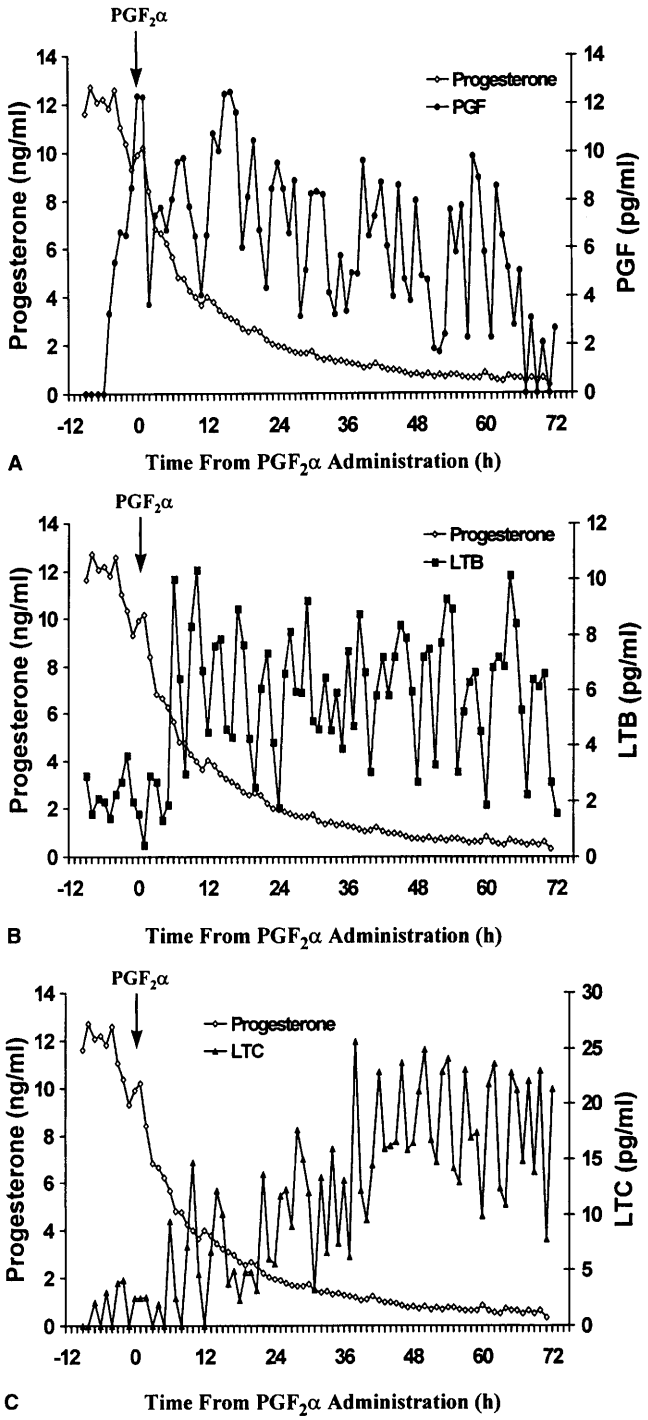

Figure 3. Mean dialysate hormone profiles of (A) PGF, (B) LTB, and (C) LTC along with the mean P_4 dialysate profile from Induced heifers. $PGF_{2\alpha}$ administration resulted in the stimulation of LTB and LTC secretion with LTC secretion being 2- to 3-fold higher than LTB.

levels was one of two heifers that did not undergo luteolysis during the sampling period, and this animal showed only a single detectable peak in OT. Plasma levels of OT (ranging from a low of 3.7 pg/ml [heifer #626] to a high of 19.5 pg/ml [heifer #634]) were detectable in all animals and remained unaltered throughout the sampling period.

Differences in the characteristics of the hormone profiles of PGF, LTB, and LTC were observed within treatment groups (Table I). The interval from the onset of P_4 decline to the peak hormone value for PGF and LTB was not different in Spontaneous heifers; however, this interval tended to be shorter ($P = 0.08$) for LTC (9.3 ± 4.5 hr) compared with LTB (35.8 ± 9.7 hr). On average, the initial peak of LTB, LTC, and PGF occurred prior to the onset of P_4 decline in Spontaneous heifers, and the mean number of hormone peaks occurring prior to the onset of P_4 decline was similar for all three AA metabolites. In Spontaneous heifers, fewer ($P < 0.05$) peaks were evident before the onset of P_4

Table I. Characteristics (Mean ± SEM) of PGF, LTB, and LTC Hormone Profiles from Spontaneous and Induced Heifers Measured by *in Vivo* Microdialysis

	Spontaneous			Induced		
	PGF	LTB	LTC	PGF	LTB	LTC
Number of heifers	4	4	3	6	6	5
Time from onset of P_4 decline to first peak (hr)[a]	−12.8 ± 8.1	−22.0 ± 6.1	−11.0 ± 8.9	10.5 ± 8.1	3.8 ± 3.8	20.0 ± 5.6
Peak level (pg/ml)	20.5 ± 4.5[b]	9.7 ± 1.7[b]	37.3 ± 8.4[c]	32.6 ± 2.4[d]	11.8 ± 1.5[e]	36.5 ± 4.6[d]
Time from onset of P_4 decline to peak level (hr)	22.8 ± 9.5	35.8 ± 9.7	9.3 ± 4.5	22.5 ± 8.8[d]	35.2 ± 10.2[d,e]	48.2 ± 8.8[e]
Time from first peak to peak level (hr)	35.5 ± 13.3[b,c]	59.8 ± 9.0[b]	20.3 ± 7.9[c]	12.0 ± 4.0	33.2 ± 10.4	28.2 ± 6.2

[a] Negative numbers indicate hours before the onset of P_4 decline.
[b,c] Values within row in the Spontaneous heifers with different superscripts are significantly different ($P < 0.05$).
[d,e] Values within row in the Induced heifers with different superscripts are significantly different ($P < 0.05$).

decline than after the decline in P_4 values for LTB (3.5 ± 2.2 vs 11.3 ± 3.0) and PGF (2.0 ± 0.9 vs 7.5 ± 1.7), whereas the mean number of peaks before and after P_4 decline were similar for LTC (5.3 ± 3.9 vs 10.7 ± 1.7). To determine the relationship between peak frequency and the onset of P_4 decline, the overall sampling period was divided into individual 12-hr intervals and the peak frequency for each interval calculated. The mean 12-hr sampling interval in which the onset of P_4 decline occurred was 3.25 ± 0.48 for Spontaneous heifers. Leukotriene C peak frequency was greater than both LTB and PGF peak frequency during the third 12-hr sampling interval, coincident with the onset of P_4 decline. Correlation analysis confirmed these data, demonstrating that the 12-hr interval with the highest LTC peak frequency was highly correlated ($r = 1.00$; $P < 0.01$) with the onset of P_4 decline. The opposite was true in the later 12-hr sampling periods, in which the peak frequency of LTB was greater ($P < 0.05$) than LTC during the fifth and sixth 12-hr sampling intervals of Spontaneous heifers.

In Induced animals, both peak LTC and PGF were higher ($P < 0.01$) than peak LTB. The interval from the onset of P_4 decline to peak LTC was greater ($P < 0.05$) than the interval to peak PGF. The mean initial peak for each hormone occurred after the decline of P_4 in Induced animals. As seen with Spontaneous heifers, the mean number of hormone peaks occurring prior to the onset of P_4 decline was similar for all three AA metabolites in Induced animals. The mean number of peaks evident before the onset of P_4 decline was lower ($P < 0.05$) than the mean number of peaks after P_4 decline in Induced heifers (0.67 ± 0.42 vs 11.0 ± 1.5, 0.00 ± 0.00 vs 14.0 ± 2.6, and 0.50 ± 0.34 vs 10.8 ± 1.6 for LTB, LTC, and PGF, respectively). The 12-hr sampling interval in which the onset of P_4 decline occurred was 1.83 ± 0.17 for Induced heifers.

Hormone profiles from one of the two Spontaneous heifers that failed to undergo luteolysis are shown in Figure 4. In this heifer, P_4 levels remained elevated. Though this heifer did have some intermittent peaks of PGF and a single OT peak, LTB and LTC were not evident until midway through Day 20 of the estrous cycle. It is possible that this heifer was just beginning to undergo luteal regression. In the remaining heifer that did not undergo luteolysis, LTB was consistently present throughout the sampling period. However, this animal did not exhibit detectable levels of LTC until midway through Day 19 of the estrous cycle and did not have detectable levels of PGF at any time.

Discussion

In the present study, we have utilized the technique of *in vivo* microdialysis to characterize the secretion of P_4, OT, and AA metabolites from the bovine CL during spontaneous and induced luteolysis. Serial microdialysis consists of inserting a length of microdialysis tubing into the tissue/organ of interest. The inside of the tubing is continuously flushed with a solution of known composition, while the outside is exposed to the surrounding tissue and its extracellular fluid. Substances produced by the tissue pass through the microdialysis tubing into the perfusion medium by simple diffusion (36–39). This technique has been used primarily to study neurotransmission (38), but recently it has also been utilized to study luteal function in cattle (18, 24, 40–44), sheep (45), pigs (23, 46), and primates (47–49). *In vitro,* microdialysis does not appear to adversely affect functionality of the CL (49).

Recovery of substances across the dialysis membrane is dependent upon several factors including flow rate, temperature, and dialysis membrane area and composition (37). Our recovery rates for P_4 (1%) and OT (0.1%) were similar to those observed by Jarry *et al.* (23) and Shaw and Britt (42), who used perfusion flow rates similar to the rate used in the present study. In other studies (24, 40, 41), recovery rates were 1%–3% for P_4, OT, and $PGF_{2\alpha}$. Though these recovery rates are substantially higher than those observed in the present study, these researchers were infusing these substances through the microdialysis tubing rather than collecting them, and they used a perfusion flow rate that was twice that used in our study.

The existence of a positive feedback loop between uterine $PGF_{2\alpha}$ and luteal OT appears to be of major importance during ovine luteolysis (50); however, the importance of a

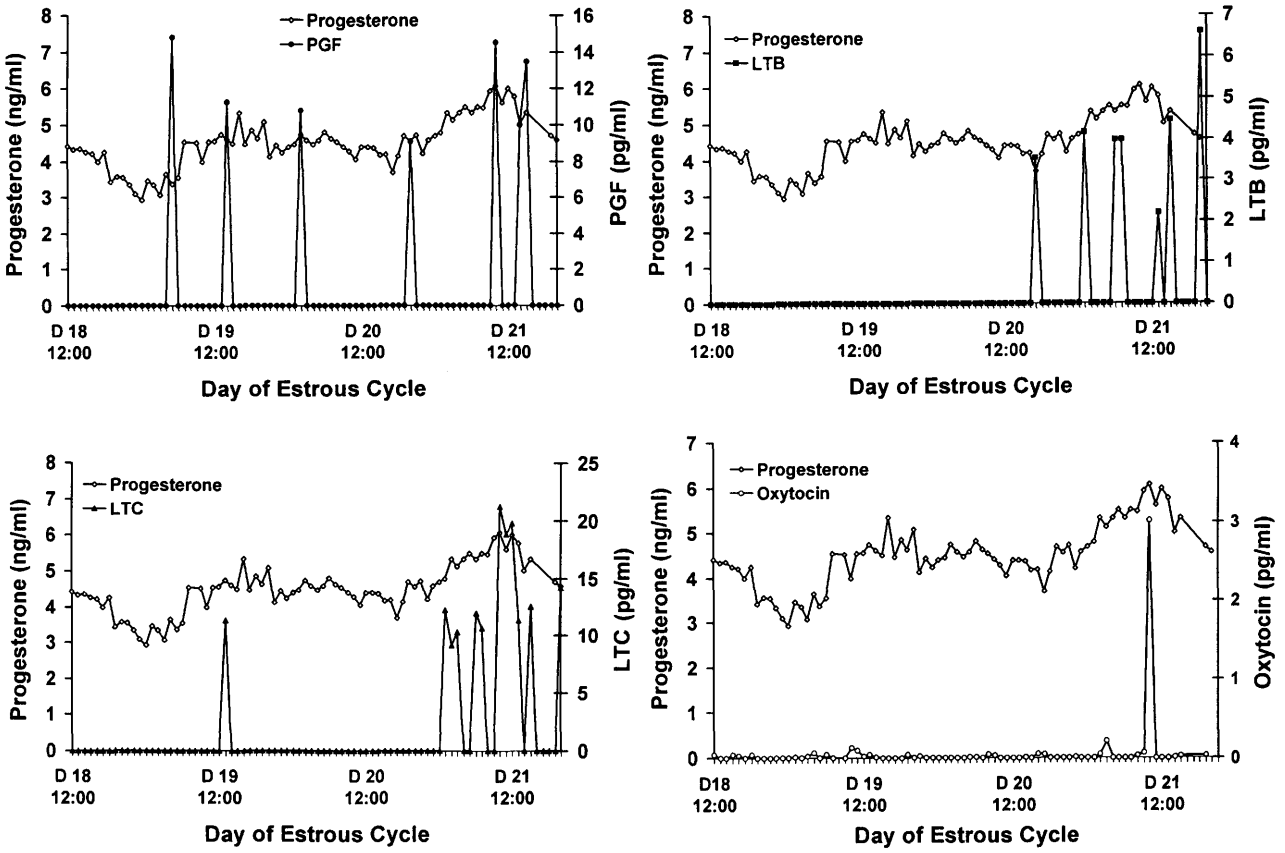

Figure 4. Dialysate hormone profiles of (A) PGF, (B) LTB, (C) LTC, and (D) OT from one of the two animals that did not undergo luteal regression during the sampling period. Though intermittent peaks of PGF and a single peak of OT were observed, LTB and LTC secretion was not evident until the middle of Day 20 of the estrous cycle, suggesting that this heifer may be just beginning to undergo luteolysis.

$PGF_{2\alpha}$-OT feedback loop in cattle remains less certain (4–7). Armstrong and Hansel (19) first indicated an inhibitory effect of OT on CL function during Days 2–6 of the estrous cycle; however, their data indicated that OT prevented normal luteal development rather than causing premature luteal regression. Although OT, injected on Days 4–6, was capable of stimulating the release of $PGF_{2\alpha}$ into the uterine vein, no changes in $PGF_{2\alpha}$ concentration in ovarian arterial blood were detected (51).

Immunocytochemical and *in situ* hybridization analysis of OT and OT mRNA contents of bovine CL demonstrate that little or no OT or OT mRNA is present during the time of luteolysis (26, 52, 53). Others have measured luteal OT content and have indicated that OT is low from Days 1–4 of the estrous cycle, increases from Days 5–12, declines rapidly from Days 13–17, and reaches a nadir from Days 18–20 of the estrous cycle (22, 25, 54). Plasma concentrations of OT parallel luteal content. Oxytocin concentrations in plasma are lowest during the time of normal luteal regression with no significant alterations evident between Days 14 and 19 of the estrous cycle (25, 55). Not only is OT low during the time of luteolysis, but specific binding sites for OT on the bovine CL are lowest on Days 15–18 of the estrous cycle (56). The *in vivo* data discussed above are reflected by *in vitro* studies utilizing microdialysis. Oxytocin release from bovine CL *in vitro* is lowest in the mid and late luteal phase (24, 41).

In the present study, only one Spontaneous and one Induced heifer exhibited detectable levels of OT in the dialysate. This may be due to the low levels of OT present during the time of normal luteal regression and to the low recovery rate of OT across the dialysis membrane. Three of the Spontaneous heifers and all of the Induced heifers except one underwent normal luteolysis in the absence of detectable levels of OT, suggesting that successful regression of the CL does not require OT. A similar conclusion was reached by Kotwica and Skarzynski (57), who demonstrated that depletion of approximately 75% of luteal OT content on Days 12 and 16 did not affect spontaneous luteolysis or the interestrous interval. The one Spontaneous heifer with a single peak of OT was one of the two animals in which luteolysis did not occur, although peaks of LTB and LTC and a small decline in P_4 suggested that luteolysis had just begun when the dialysis was stopped (Fig. 4).

We did not observe significant changes in plasma concentrations of OT in either Spontaneous or Induced heifers in the present study. This was not completely unexpected since others (22, 25) also failed to detect alterations in jugular plasma of normal heifers from Days 14 to 19 of the estrous cycle. Thus, the data from the present study, as well

as from previous work, fail to substantiate an essential role for luteal OT in luteal regression in cattle. Recent evidence suggests that OT administered directly into the CL by an MDS may actually be luteotrophic *in vivo,* especially in the early and mid-luteal phases (18).

An increasing amount of evidence suggests that AA and its 5-LO metabolites may be key factors in luteal regression (5, 6). Hansel and Fortune (28) demonstrated that AA infusion into the ovarian arteries of heifers in which all connections between the ovary and the uterine horn ipsilateral to the CL were severed caused luteal regression. Therefore, it was evident that AA could stimulate luteolysis in the absence of uterine $PGF_{2\alpha}$. However, the mechanisms by which AA induced luteal regression remained unclear. In a series of *in vitro* and *in vivo* experiments, Milvae and coworkers (29) provided evidence that products of the 5-LO pathway of AA metabolism were involved in luteolysis. These authors showed that the bovine CL contains large quantities of 5-hydroxyeicosatetraenoic acid (5-HETE), and that addition of 5-HETE to luteal cell cultures resulted in a decrease in P_4 without altering $PGF_{2\alpha}$ secretion. Twice daily intrauterine infusions of NDGA, a lipoxygenase inhibitor, from Days 14 to 18 of the estrous cycle delayed luteolysis and extended the interestrous interval (29). A similar result was observed in sheep (30, 31). Intrauterine infusions of NDGA to ewes on Days 10–14 delayed luteolysis and increased the estrous cycle length while reducing peripheral plasma concentrations of 13,14-dihydro-15-keto-$PGF_{2\alpha}$ and OT (30). These authors further demonstrated that NDGA infusions inhibit $PGF_{2\alpha}$-stimulated OT secretion in sheep (31). These results suggest that 5-LO metabolites of AA are important components of luteal regression.

One class of 5-LO metabolites of AA, the leukotrienes, has been implicated as a potential mediator of luteal regression. Steadman and Murdoch (32) demonstrated that administration of $PGF_{2\alpha}$ to ewes on Day 10 of the estrous cycle stimulated the secretion of LTB_4 within 2 hr, before either functional or structural luteolysis was evident. A similar role in luteolysis for LTB_4 has been suggested in mares, where addition of $PGF_{2\alpha}$ to diestrous equine luteal cells in culture increased LTB_4 production (33).

In the present study, we have demonstrated that the bovine CL secretes both LTB and LTC during spontaneous luteolysis and in response to luteolytic injections of $PGF_{2\alpha}$. Dialysate peaks of LTB and LTC, as well as PGF, were present with increasing frequency and at higher levels as P_4 declined. There were some interesting differences between the hormone profiles of LTB and LTC. Both of these LTs were present in the dialysate prior to the onset of P_4 decline. In Spontaneous heifers, LTB was present for a longer period of time and at fairly consistent levels, whereas LTC secretion was focused near the onset of P_4 decline and exhibited significantly higher levels than LTB. The high levels of LTC, the timing of its secretion with reference to the onset of P_4 decline (Fig. 2), and the large numbers of its receptors found in large and small luteal cells (58, 59) all suggest that it may play a direct role in luteal regression. Preliminary results from our laboratory (Blair and Hansel, unpublished data) support this hypothesis. Addition of LTC_4 to luteal cell cultures at concentrations of 10 and 50 μM dramatically decreased P_4 production at 12 (33.76, 20.96, and 11.36 ng/50,000 cells for 0, 10, and 50 μM LTC_4, respectively), 18 (34.56, 22.16, and 8.24 ng/50,000 cells for 0, 10, and 50 μM LTC_4, respectively) and 24 (41.76, 21.52, and 8.48 ng/50,000 cells for 0, 10, and 50 μM LTC_4, respectively) hr of culture. Since LTB_4 is a potent chemoattractant for immunological cells (60–62), it is possible that LTB_4 may play an indirect role in luteolysis through chemoattraction of immune cells.

The results of the present study also concur with those of Steadman and Murdoch (32) and Watson (33) in that $PGF_{2\alpha}$ administration stimulated the secretion of LTs. Although peak heights for LTB and LTC were unaltered, the number of peaks was substantially higher after $PGF_{2\alpha}$ administration compared with before $PGF_{2\alpha}$ treatment. Thus, it is evident that luteal secretion of LTs is enhanced by an exogenous luteolytic dose of $PGF_{2\alpha}$, further suggesting the involvement of LTs in bovine luteolysis.

In summary, the present study demonstrates that the bovine CL produces substantial amounts of LTB and LTC during spontaneous luteolysis as well as in response to exogenous $PGF_{2\alpha}$. The hormone profiles of PGF and LTB indicate that their production may be a consequence of luteolysis. However, the similarity in the mean number of LTC peaks before and after the onset of P_4 decline in Spontaneous animals suggests that LTC may play a more direct role in luteolysis. We did not find detectable levels of OT secreted by the CL in most of the animals studied. Together, data from the present study and results from previous work suggest that 5-LO metabolites of AA metabolism, specifically LTB and LTC, may play key roles in regression of the bovine CL, and fail to support the concept that OT of luteal origin plays an essential role.

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