BIOLOGY OF REPRODUCTION 58, 346–352 (1998)

Prostaglandin $F_{2\alpha}$ Regulates Distinct Physiological Changes in Early and Mid-Cycle Bovine Corpora Lutea[1]

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ABSTRACT

Prostaglandin (PG) $F_{2\alpha}$ is the primary luteolysin in most species. A single treatment with $PGF_{2\alpha}$ will cause regression of the mid-cycle but not the early-cycle (Days 1–5 after estrus) bovine corpus luteum (CL) despite the presence of similar concentrations of high-affinity $PGF_{2\alpha}$ receptors (FP receptors). This study was designed to determine whether $PGF_{2\alpha}$ activated similar intracellular processes in early- and mid-cycle CL. Cows received saline or 25 mg $PGF_{2\alpha}$ injection (i.m.; n = 6/group) on Day 4 or 11 after onset of the LH surge (induced by GnRH injection), and CL were collected at 4 h after treatment. As expected, CL volumes and luteal weights were not different at 4 h after $PGF_{2\alpha}$ treatment. Luteal vitamin C concentration and steady-state concentrations of mRNA for 3β-hydroxysteroid dehydrogenase and for FP receptor were decreased by 4 h in both Day 4 and 11 CL treated with $PGF_{2\alpha}$ ($p < 0.05$). These results demonstrate clear actions of $PGF_{2\alpha}$ in the early CL. In contrast, steady-state concentrations of mRNA encoding PG G/H synthase-2 (PGHS-2) were increased by treatment with $PGF_{2\alpha}$ in mid-cycle CL but decreased by $PGF_{2\alpha}$ in early-cycle CL ($p < 0.05$). In addition, treatment of mid-cycle but not early-cycle cows with $PGF_{2\alpha}$ decreased luteal and serum progesterone concentrations by 4 h ($p < 0.05$). In summary, $PGF_{2\alpha}$ clearly exerts actions in both early- and mid-cycle bovine CL. The lack of $PGF_{2\alpha}$-induced luteolysis in the early CL may be due to specific changes in gene expression, especially PGHS-2, that may prevent intraluteal $PGF_{2\alpha}$ production and possibly other key luteolytic processes.

INTRODUCTION

Luteolysis is a complex process involving changes in expression of multiple genes in at least 3 cell types (large and small steroidogenic cells and endothelial cells) [1–11]. There are reports that either natural luteolysis or treatment with $PGF_{2\alpha}$ decrease mRNA encoding gene products that regulate steroidogenesis [1–4], increase mRNA for stress-related or apoptotic genes [5–7], and increase mRNA for factors involved in immune response [8–10]. Recently, it was found that $PGF_{2\alpha}$ also increased mRNA encoding enzymes that stimulate $PGF_{2\alpha}$ production in the ovine corpus luteum (CL) [12]. In addition to regulation of gene expression, $PGF_{2\alpha}$ also causes acute morphological and physiological changes in luteal cells such as decreased membrane fluidity [13], depletion of antioxidants from the CL [14], and increased activity of phospholipases and proteolytic enzymes [15]. The combination of these $PGF_{2\alpha}$-induced changes results in cessation of luteal progesterone (P_4) production and involution of luteal tissue. $PGF_{2\alpha}$ exerts its effects by binding to a plasma membrane receptor (FP receptor), a G-protein-coupled receptor with seven trans-membrane domains [16]. Although mechanisms responsible for $PGF_{2\alpha}$-induced luteolysis are not completely defined, there is evidence supporting the involvement of the calcium/protein kinase (PK)C effector system in $PGF_{2\alpha}$ actions [17].

For those species in which $PGF_{2\alpha}$ is luteolytic in vivo, administration of $PGF_{2\alpha}$ or its analogues in the early luteal phase does not cause luteal regression. For example, in cattle the CL is unresponsive to an injection of $PGF_{2\alpha}$ on or before Day 5 of the cycle [18–21]. This insensitivity to $PGF_{2\alpha}$ injection is seen before Day 8 of the 20-day luteal phase in marmoset monkeys [22], before Day 13 in pigs [23], and before Day 4 in pregnant or pseudopregnant rats [24]. Although little is known of the mechanisms responsible for this insensitivity of the early CL to the luteolytic action of $PGF_{2\alpha}$, it is clear that it is not due to lack of high-affinity FP receptors in cattle [25–27] or rats [24]. In pigs, there seems to be an increase in numbers of FP receptors on Day 13; however, numerous high-affinity FP receptors (~1 million/cell) were also detected in early CL [28].

We hypothesized that lack of luteolysis of early bovine CL (before Day 5 of the estrous cycle) after treatment with $PGF_{2\alpha}$ may be due to altered $PGF_{2\alpha}$-induced gene expression in the early CL. This study was designed to determine whether $PGF_{2\alpha}$ activated similar intracellular processes in early- and mid-cycle CL. The cow is an excellent animal model for this study because the stage of CL that can or cannot be regressed by treatment with $PGF_{2\alpha}$ is well defined [18–21], and a similar number of high-affinity FP receptors are present in the CL of both stages [25–27].

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals used in this study, unless otherwise specified, were purchased from Sigma (St. Louis, MO). T7 RNA polymerase and restriction enzymes were purchased from Promega (Madison, WI). *Taq* DNA polymerase, SuperScript II RNase H minus M-MLV reverse transcriptase, and a 1-kilobase DNA ladder were from Gibco/BRL (Gaithersburg, MD). Magnetight oligo(dT) particles were purchased from Novagen (Madison, WI). GnRH (Cystorelin) was donated by Rhone Merieux Inc. (Overland Park, KS). $PGF_{2\alpha}$ (Lutalyse) was donated by Pharmacia-Upjohn Co. (Kalamazoo, MI).

Animals

Nonlactating dairy cows at the University of Wisconsin were used in this study. Follicular growth and time of ovulation were synchronized in all cows by using a recently developed protocol [29]. Briefly, cows at different stages of the estrous cycle each received an i.m. injection of 100 μg of GnRH to ovulate follicles with ovulatory capacity and thus to initiate a new follicular wave. Seven days later, an i.m. injection of 25 mg of $PGF_{2\alpha}$ was given to regress

Accepted September 11, 1997.
Received July 16, 1997.
[1]This research was supported by NIH grant HD-32623 and the Wisconsin Experiment Station.
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old (ovulated before GnRH) and/or new (ovulated in response to GnRH injection) CL. The LH surge was induced by an injection of GnRH (100 μg), at 48 h after $PGF_{2\alpha}$ treatment, to ovulate the dominant follicle. This protocol synchronized follicular growth in more than 95% of cows [29] and induced ovulations within an 8-h period (between 24 and 32 h after GnRH injection as monitored by transrectal ultrasound using an Aloka [Tokyo, Japan] 500V ultrasound machine with a 7.5-mHz linear array transducer). Once ovulation was confirmed, cows were randomly assigned to one of four treatment groups (2 × 2 factorial design, n = 6/group). Cows in groups I and II received $PGF_{2\alpha}$ (25 mg) or saline injection (i.m.), respectively, on Day 4 after GnRH injection. Cows in groups III and IV received $PGF_{2\alpha}$ or saline injection, respectively, on Day 11 after GnRH injection. Four h after $PGF_{2\alpha}$ injection, ovaries were removed by colpotomy, and CL were enucleated and immediately frozen in liquid nitrogen. In a separate experiment, eight more cows were randomly assigned to receive saline or $PGF_{2\alpha}$ injection (n = 4/group) on Day 4 and again on Day 11 after GnRH injection (saline-saline or $PGF_{2\alpha}$-$PGF_{2\alpha}$). The CL in these cows remained intact throughout the time of the study. For both studies, daily ultrasonography and blood sampling were performed from the day of ovulation and continued until two days after colpotomy or Day 14 of the cycle (CL intact groups). In addition, blood samples were collected at 0, 2, 4, and 8 h after saline or $PGF_{2\alpha}$ injection. All animal procedures were approved by the Research Animal Resources Center of the University of Wisconsin-Madison.

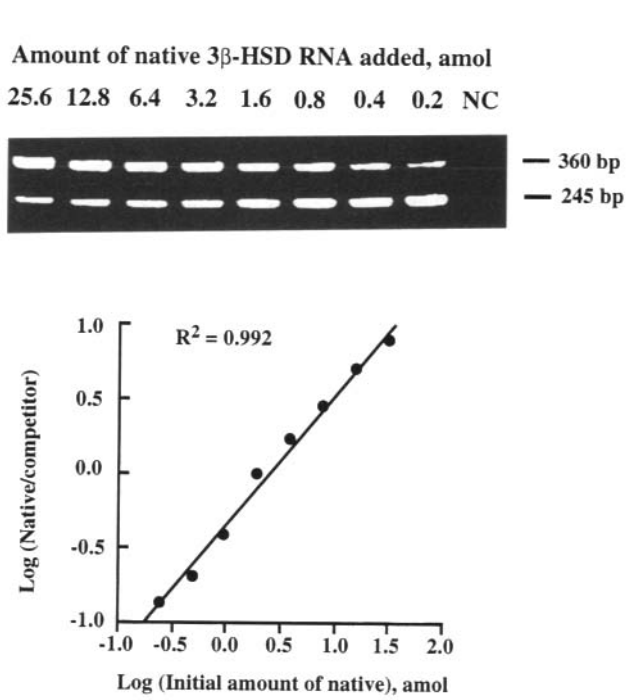

FIG. 1. Standard-curve method QC-RT-PCR for quantification of steady-state concentrations of mRNA for 3β-HSD. **A**) A representative photograph of PCR product stained with ethidium bromide containing native (upper band) and competitor (lower band) DNA. **B**) A standard curve produced from **A**.

Isolation and Quantification of mRNA

Isolation of mRNA from luteal tissue has been described previously [25] with the exception that Magnetight oligo(dT) particles from Novagen were used. Quantitative, competitive (QC) reverse transcription (RT)-polymerase chain reaction (PCR; QC-RT-PCR) procedures using standard-curve methodology for the determination of FP receptor and PG G/H synthase-2 (PGHS-2) mRNA were developed and validated in previous studies [25, 30]. A similar QC-RT-PCR procedure was developed to quantify 3β-hydroxysteroid dehydrogenase (3β-HSD) in this study (Fig. 1). Briefly, specific primers were designed according to published bovine 3β-HSD cDNA sequences [31]. A DNA fragment of 360 base pairs (bp) was amplified from bovine mRNA by RT-PCR and subcloned into a PCR II vector (Invitrogen, San Diego, CA), which represented plasmid containing "native" sequence. An internal region of 115 bp was deleted from the 360-bp DNA fragment by PCR using a procedure similar to that described by Gilliland et al. [32]. The PCR product was then subcloned into PCR II vector, representing plasmid containing "competitor" sequence. Both plasmids were linearized with *Bam*HI restriction enzyme and in vitro-transcribed using T7 RNA polymerase. RNase-free DNase I (Boehringer Mannheim, Indianapolis, IN) was used to remove plasmid DNA from the RNA pool. In vitro-transcribed RNA was precipitated twice with 2.5 volumes of 100% ethanol and 0.3 M sodium acetate (pH 4.0) after phenol-chloroform extraction. The concentration of RNA was determined by 260 nm absorbance. RNA was aliquoted and stored at −80°C until used. The standard QC-RT-PCR assay contained 2 amol competitor RNA and 8 serial dilutions of native RNA (from 25.6 to 0.2 amol) or mRNA from unknown samples in a final volume of 20 μl of reverse-transcription mix (single-strength first-strand synthesis buffer, 10 mM dithiothreitol [DTT], 0.2 mM deoxynucleoside 5′-triphosphates (dNTPs), and 40 U SuperScript II M-MLV reverse transcriptase). Reverse transcription, carried out at 42°C for 60 min, was followed by heating at 95°C for 10 min. Half of the RT products were amplified by 30 cycles of PCR (95°C: 30 sec, 57°C: 30 sec, and 72°C: 30 sec) in a PCR solution (single-strength PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 μM/each of primers, and 0.5 U of *Taq* polymerase) and then incubated at 72°C for 5 min. Semi-quantitative RT-PCR using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal control was used to quantify steroidogenic acute regulatory protein (StAR) mRNA. The conditions for semi-quantitative RT-PCR using GAPDH as an internal control were similar to those described previously [25]. PCR products were separated on a 5% acrylamide gel, stained with ethidium bromide, and analyzed using Collage computer software (Fotodyne, Hartland, WI). Sequences for all the primers used in this study are listed in Table 1.

Ascorbic Acid Assay

Luteal ascorbic acid content was measured by a procedure similar to that used by Arakawa et al. [33], using 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline, BP) as a reagent. Luteal tissues were ground into powder under liquid nitrogen and weighed. About 50 mg of tissue was homogenized in 1 ml of 5% trichloroacetic acid (TCA) solution using a Dounce homogenizer. The homogenate was centrifuged at 16 000 × *g* for 5 min to remove protein aggregate and cell debris, and the solution was recovered and stored at −20°C for later determination of ascorbic acid and P_4 concentrations. In a 96-well plate, 50 μl of sample solution extracted in 5% TCA was added to each well. To each well, 50 μl of 100% ethanol (EtOH), 25 μl of 0.4%

TABLE 1. Sequences for primers used in this study.

Gene	Primer name	Sequence	Species	Ref.
FP receptor	A0035: A0034:	5′ GTAAAAAGGGTTTCACAGG 3′ 5′ CAAAGACTGGGAAGATAGGTT 3′	Bovine	[16]
PGHS-2	bPGS2A: bPGS2B:	5′ AGGTGTATGTATGAGTG-TAGGA 3′ 5′ GTGCTGGGCAAAGAATGCAA 3′	Bovine	[25]*
3β-HSD	3bHSD-1: 3bHSD-2:	5′ TGTTGGTGGAGGAGAAGG 3′ 5′ GGCCGTCTTGGATGATCT 3′	Bovine	[31]
GAPDH	GP1: GP2:	5′ TGTTCCAGTATGATTCCACCC 3′ 5′ TCCACCACCCTGTTGCTGTA 3′	Bovine	[25]*
StAR	bSTAR-1: bSTAR-2:	5′ CCTCTCTACAGCGACCAA 3′ 5′ TCGTGAGTGATGACCGTG 3′	Bovine	[52]

* The PGHS-2 and GAPDH bovine primer sequences reported in [25] were designed from our unpublished partial cDNA sequences.

H_3PO_4-EtOH, 50 μl of 0.5% BP-EtOH, and 25 μl of 0.03% $FeCl_3$-EtOH were added in order. The solution was allowed to stand at 37°C for 60–90 min for the Fe^{2+}-BP complex to develop. The absorbance of the color solution was read at 490 nm in an enzyme immunoassay (EIA) plate reader (Bio-Tek Instruments, Model EL310; Fisher, Pittsburgh, PA). The assay sensitivity was 1 μg/ml with intra- and interassay coefficients of variance of 6.6% and 14.6%, respectively.

P_4 Assay

P_4 was extracted from serum using a double-extraction procedure as described [34]. Extraction efficiency was evaluated by extracting charcoal-stripped serum containing two different concentrations of P_4. This procedure recovered about 85% (from 80% to 95%) of the P_4 in the serum.

The extracted serum P_4 was reconstituted in same volume of assay buffer (40 mM 3[*N*-morpholino]propanesulfonic acid [MOPS], 0.12 M NaCl, 10 mM EDTA, 0.1% gelatin, 0.05% Tween 20, and 0.005% chlorhexidine digluconate, pH 7.4) and directly used for P_4 assay. One part of TCA-extracted luteal P_4 solution was mixed with 49 parts of assay buffer (1:50 dilution) before ELISA was performed. Luteal and serum P_4 was determined by a competitive ELISA procedure as described [34]. Briefly, 100 μl of primary antibody (1:150 000; mouse anti-P_4 monoclonal antibody; Biostride Inc., Palo Alto, CA) was added to a 96-well plate precoated with 100 μl of goat-anti-mouse antibodies (1 ng/ml; Calbiochem, San Diego, CA) and incubated for 90 min at room temperature. After excess primary antibody was washed off, 100 μl of samples were added to the plate and incubated for another 90 min at room temperature. Fifty microliters of horseradish peroxidase-conjugated P_4 (made in our laboratory) was added to each well to compete for the primary antibody for 90 min at room temperature. The plate was then washed 4 times with washing buffer (20 mM MOPS and 0.05% Tween-20, pH 7.2). Substrate solution (125 μl; 50 mM sodium acetate [pH 4.4], 0.5 M H_2O_2, and 20 mg/ml 3,3′,5,5′-tetramethyl benzidine) was added to each well and incubated at 37°C for 15 min with shaking. Color development was terminated by adding 50 μl of stop solution (0.5 M H_2SO_4) to each well, and optical density was determined by reading absorbance at 450 nm in an EIA plate reader. The sensitivity (80% bound) of P_4 assay was 0.2 ng/ml, and the intra- and interassay CV were 5.2% and 9.6%, respectively.

Statistical Analysis

Paired *t*-tests were used for comparison of serum P_4 concentration and luteal diameter within a day. Analysis of variance was used to compare mRNA concentrations, luteal vitamin C content, luteal weights, luteal volumes, and luteal P_4 concentration using general linear model procedures of Statistical Analysis Systems. Difference between least-square means were evaluated by Fisher's protected least significant difference.

RESULTS

Effect of $PGF_{2\alpha}$ on CL Volume, Luteal Weight, Luteal Ascorbic Acid Content, and Serum P_4 Concentration

Mid-cycle (Day 11) CL were larger, heavier, and secreted more P_4 into circulation than early-cycle (Day 4) CL (Table 2). As expected, neither CL volume nor luteal weight was changed by $PGF_{2\alpha}$ at 4 h after injection on either Day 4 or Day 11 as compared to those of the saline group (Table 2). In saline-treated groups, Day 11 CL had higher luteal P_4 concentrations per gram of tissue than did Day 4 CL (Table 2). Luteal P_4 concentrations were significantly decreased at 4 h after treatment with $PGF_{2\alpha}$ in Day 11 CL compared to saline-treated group (Table 2), and this decrease was mirrored by decreased serum P_4 concentration at 4 h after $PGF_{2\alpha}$ treatment (before removal of CL, Table 2). On Day 4, both luteal and serum P_4 concentrations were not different between the $PGF_{2\alpha}$- and saline-treated groups (Table 2). In intact cows, there was no difference in serum

TABLE 2. Comparison of $PGF_{2\alpha}$ action on Day 4 and Day 11 CL at 4 h after treatment.

Treatment	n	Luteal vol (mm^3)	Luteal weight (g)	Luteal P_4 (μg/g)	Serum P_4 (ng/ml)	Luteal vitamin C (μg/g tissue)
Day 4						
Saline	6	1118 ± 83[a]	1.00 ± 0.14[a]	1.08 ± 0.24[a]	0.58 ± 0.08[a]	1284 ± 265[a]
$PGF_{2\alpha}$	6	1068 ± 125[a]	1.12 ± 0.13[a]	1.01 ± 0.18[a]	0.46 ± 0.05[a]	862 ± 76[b]
Day 11						
Saline	6	5931 ± 776[b]	5.04 ± 1.16[b]	2.23 ± 0.73[b]	3.88 ± 0.29[c]	1743 ± 183[a]
$PGF_{2\alpha}$	6	5334 ± 481[b]	4.73 ± 1.08[b]	0.63 ± 0.13[a]	1.94 ± 0.22[b]	874 ± 82[b]

[a,b] Value within a column with different superscript is different ($p < 0.05$).

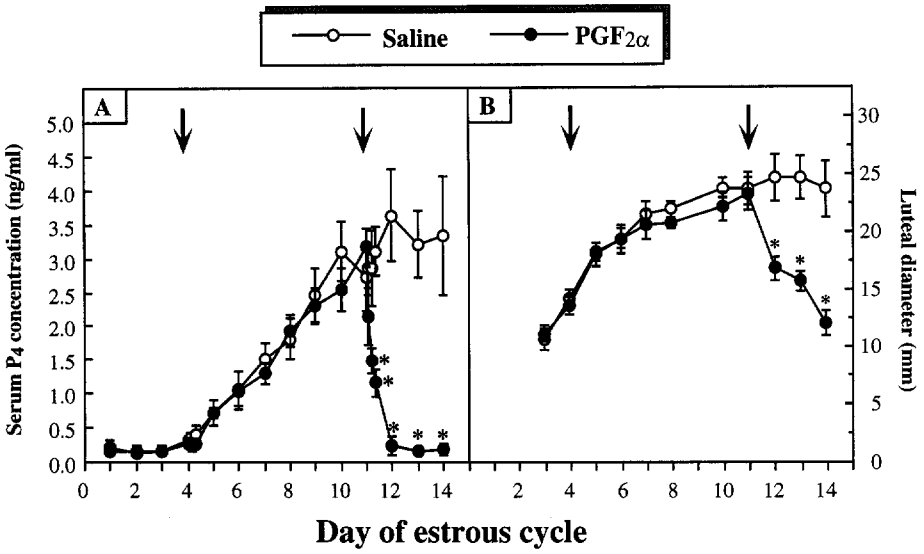

FIG. 2. Serum P_4 concentration (**A**) and size of CL (**B**) after $PGF_{2\alpha}$ treatment of early- and mid-cycle cows. Arrows indicate time of saline or $PGF_{2\alpha}$ injection. * Difference from control group (saline, $p < 0.05$).

P_4 concentrations and luteal diameters between saline- and $PGF_{2\alpha}$-treated cows before Day 11, and both were decreased by treatment with $PGF_{2\alpha}$ on Day 11 (Fig. 2, A and B).

Luteal ascorbic acid concentrations did not differ in Day 4 and Day 11 CL at 4 h after saline treatment (Table 2). Prostaglandin $F_{2\alpha}$ decreased luteal ascorbic acid when administered to cows on either Day 4 or Day 11 (Table 2).

Effect of $PGF_{2\alpha}$ on Gene Expression in the CL

Steady-state concentrations of mRNA encoding StAR were highest in the CL of Day 11 saline-treated cows (Fig. 3A). $PGF_{2\alpha}$ decreased mRNA for StAR in Day 11 CL, but this decrease was not statistically significant (Fig. 3A, $p = 0.08$). In Day 4 CL, there was no difference in StAR mRNA between $PGF_{2\alpha}$- and saline-treated cows ($p > 0.1$). Steady-state concentrations of mRNA for 3β-HSD in the saline-treated cows on Days 4 and 11 were not different (Fig. 3B). Treatment of cows with $PGF_{2\alpha}$ significantly decreased mRNA encoding 3β-HSD within 4 h regardless of the age of the CL (Fig. 3B). Steady-state concentrations of mRNA encoding FP receptor in the saline-treated cows on Days 4 and 11 were not different (Fig. 4A). Treatment of cows with $PGF_{2\alpha}$ significantly decreased FP receptor mRNA by 4 h in both Day 4 and Day 11 CL compared to those of saline-treated cows (Fig. 4A). Basal concentrations

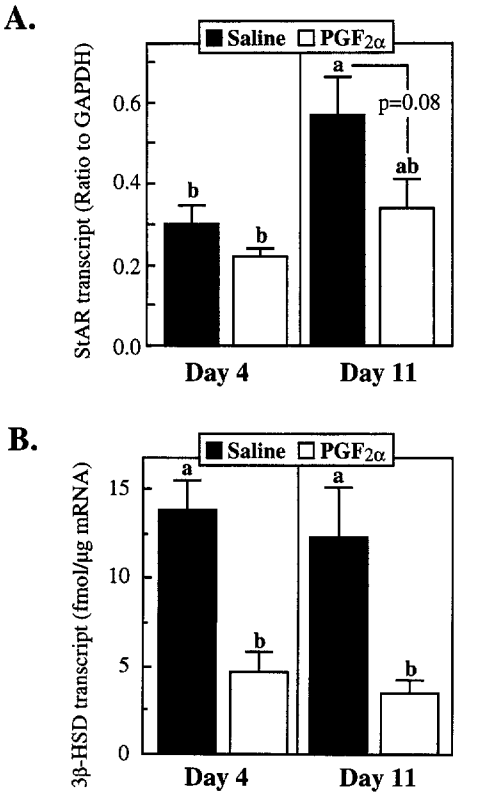

FIG. 3. Steady-state concentrations of mRNA encoding StAR (**A**) and 3β-HSD (**B**) in Day 4 and Day 11 CL collected at 4 h after saline or $PGF_{2\alpha}$ injection. Different letters within the same panel indicate differences ($p < 0.05$).

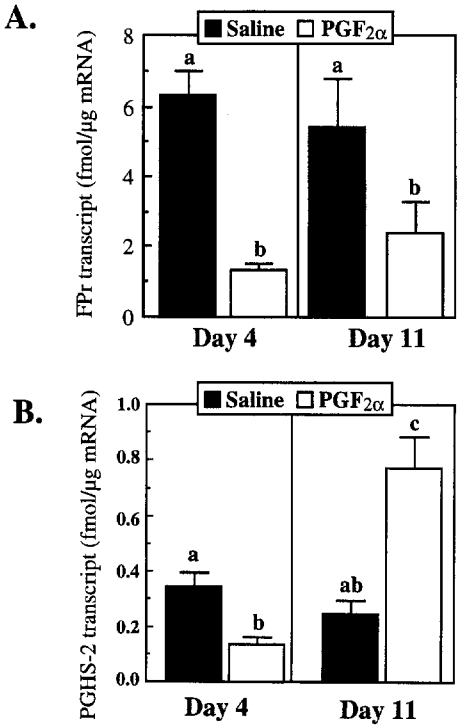

FIG. 4. Steady-state concentrations of mRNA encoding FP receptor (**A**) and PGHS-2 (**B**) in Day 4 and Day 11 CL collected at 4 h after saline or $PGF_{2\alpha}$ injection. Different letters within the same panel indicate differences ($p < 0.05$).

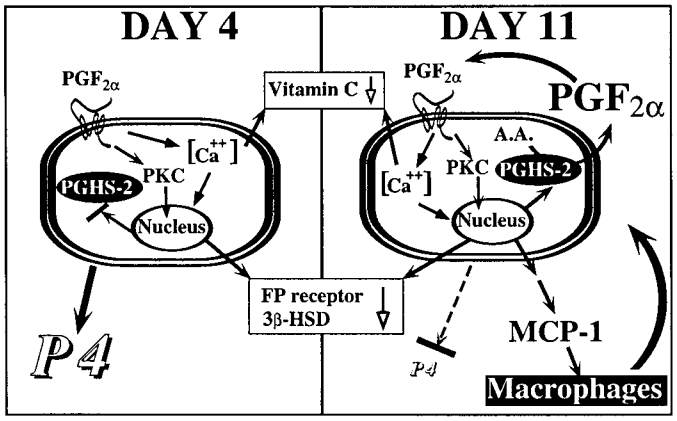

FIG. 5. Conceptual model depicting distinct gene expression in response to $PGF_{2\alpha}$ treatment in early- (Day 4) and mid-cycle (Day 11) bovine CL. A.A., arachidonic acid. See text for detailed description.

of mRNA encoding PGHS-2 were detected in both Day 4 and Day 11 CL of saline-treated cows (Fig. 4B). Treatment of cows with $PGF_{2\alpha}$ increased steady-state concentrations of mRNA for PGHS-2 in Day 11 CL but significantly decreased PGHS-2 mRNA in Day 4 CL (Fig. 4B).

DISCUSSION

The results presented in this study add to our understanding of $PGF_{2\alpha}$ actions in the early CL (unresponsive to $PGF_{2\alpha}$-induced luteolysis) and the mid-cycle CL (responsive to $PGF_{2\alpha}$-induced luteolysis). It was discovered, soon after $PGF_{2\alpha}$ had been identified as the primary luteolysin in ruminants, that $PGF_{2\alpha}$ was ineffective for synchronizing estrus in cattle in the early estrous cycle [18–21]. The mechanism responsible for the unresponsiveness of the early CL to $PGF_{2\alpha}$ is not yet clear, but the unresponsiveness does not appear to be due to a lack of high-affinity FP receptors [25–28].

It is possible that $PGF_{2\alpha}$ may not reach the early CL because blood flow is shunted toward the uterus and away from the ovary during this time [35]. The current results give clear evidence that $PGF_{2\alpha}$ reaches the early CL and exerts physiological actions in the early CL. First, $PGF_{2\alpha}$ depleted luteal ascorbic acid content in both Day 4 and Day 11 CL. The acute depletion of luteal ascorbic acid by $PGF_{2\alpha}$ has been previously demonstrated in CL of the rat and pig [14, 36, 37]. Second, steady-state concentrations of mRNA encoding 3β-HSD were decreased by $PGF_{2\alpha}$ treatment in Day 4 as well as Day 11 CL. Previous studies have shown that 3β-HSD mRNA was acutely decreased by $PGF_{2\alpha}$ in mid-cycle CL [1, 3]. Finally, FP receptor mRNA was decreased by treatment with $PGF_{2\alpha}$ in Day 4 and Day 11 CL. The effect of $PGF_{2\alpha}$ on inhibition of mRNA for FP receptor has been observed in luteal cells of ruminants both in vivo ([38, 39], unpublished results) and in vitro (unpublished results), although the physiological significance of this effect is not clear. Thus, these four clear actions of $PGF_{2\alpha}$ on the Day 4 CL indicate that $PGF_{2\alpha}$ given as an i.m. injection in cows on Day 4 of the estrous cycle arrives at the CL, binds to the FP receptor, and initiates at least part of the cascade of physiological events that leads to luteolysis. However, a single injection of $PGF_{2\alpha}$ did not complete the luteolytic process in Day 4 CL, as demonstrated by a continued increase in serum P_4 concentrations and size of CL in our control experiment.

The current concept of luteolysis has focused on the direct action of $PGF_{2\alpha}$ on large steroidogenic luteal cells. These large luteal cells contain the high-affinity FP receptors [27] and are likely to be the target for the initial actions of $PGF_{2\alpha}$ in the CL [17]. Binding of $PGF_{2\alpha}$ increases free intracellular calcium [40, 41] and activates PKC [17]. Other intracellular effectors, such as mitogen-activated protein (MAP) kinase, may also be activated [42]. Numerous changes in cellular physiology, including changes in gene expression, follow binding of $PGF_{2\alpha}$ to luteal cells. We previously reported that $PGF_{2\alpha}$ up-regulates the expression of PGHS-2 mRNA and protein in ovine large luteal cells by activating the free intracellular calcium/PKC effector system [12]. The induction of PGHS-2 resulted in increased $PGF_{2\alpha}$ production by large luteal cells [12]. Thus, in vitro data indicate that there exists a positive feedback loop within large luteal cells such that a small amount of $PGF_{2\alpha}$ would cause production of high local concentrations of $PGF_{2\alpha}$ within luteal tissue [12]. In the current study, we also found that this positive feedback loop was activated by $PGF_{2\alpha}$ in the mid-cycle bovine CL, as evidenced by a more than 3-fold increase in PGHS-2 mRNA by 4 h after $PGF_{2\alpha}$ treatment. Interestingly, $PGF_{2\alpha}$ did not increase PGHS-2 mRNA in the early CL, and actually resulted in a statistically significant decrease in PGHS-2 mRNA. It is not yet clear whether intraluteal production of $PGF_{2\alpha}$ is essential for luteolysis; however, it appears that this autoamplification cascade, as evidenced by PGHS-2 expression, is not induced by $PGF_{2\alpha}$ in the early CL.

The importance of intraluteal $PGF_{2\alpha}$ production in luteolysis is suggested by a variety of indirect evidence. The short half-life of $PGF_{2\alpha}$ requires that a single exposure to $PGF_{2\alpha}$ must be sufficient to initiate the full luteolytic cascade. Although single injections of $PGF_{2\alpha}$ do not cause the early CL to regress, multiple injections of $PGF_{2\alpha}$ have led to regression of the early CL in a few cows [20, 43]. Similarly, in pigs the normally unresponsive CL (< Day 12) can be induced to regress if 12 injections of $PGF_{2\alpha}$ are administered from Day 5 to Day 10 of the estrous cycle [44]. In animals with a mid-cycle CL and a functional $PGF_{2\alpha}$ autoamplification system, there may be little need for multiple injections because a single injection may cause production of high amounts of intraluteal $PGF_{2\alpha}$ with a lag time of 4–12 h after the initial $PGF_{2\alpha}$ treatment [12]. Physiological studies suggest that continuous infusion or multiple pulses of $PGF_{2\alpha}$ are required to induce functional regression of CL under normal conditions [45–49]. Thus, the lack of intraluteal production of $PGF_{2\alpha}$ as an additional source of $PGF_{2\alpha}$ may result in the resistance of early CL to a single injection of $PGF_{2\alpha}$.

In Figure 5 we have attempted to provide a simplified conceptual model for possible differences in regulation in the early- and mid-cycle CL. Acquisition of high-affinity FP receptors occurs by about Day 2 after ovulation, and after this time the early- and mid-cycle CL have FP receptors that are likely to activate similar intracellular effectors such as calcium/PKC. These intracellular effectors seem to acutely regulate certain processes similarly in early- and mid-cycle CL, such as depletion of vitamin C and inhibition of expression of 3β-HSD and FP receptor. Other genes or intracellular processes may be regulated by $PGF_{2\alpha}$ in the mid-cycle CL but not the early CL as evidenced by expression of PGHS-2 in this study. The mRNA for StAR appeared to be differentially regulated by $PGF_{2\alpha}$ in the mid-cycle and early CL, although this difference was not as statistically impressive. A decrease in StAR may be essential for $PGF_{2\alpha}$ action on luteal P_4 production. Previous stud-

ies have shown that acute changes in steroidogenesis appear to be primarily associated with changes in active StAR protein and not changes in activities of other steroidogenic enzymes [50]. In contrast, although $PGF_{2\alpha}$ decreases mRNA for 3β-HSD, there do not appear to be concomitant changes in 3β-HSD protein [51]. This suggests that changes in mRNA for StAR but not 3β-HSD may be important in the anti-steroidogenic effects of $PGF_{2\alpha}$; however, further studies that evaluate changes in active StAR and 3β-HSD protein are still required to test this hypothesis. In addition to its effect on large luteal cells, $PGF_{2\alpha}$-induced luteolytic effects may involve changes in intercellular communication between large luteal cells, small luteal cells, and nonsteroidogenic cells such as endothelial and immune cells [8, 11]. We recently found that $PGF_{2\alpha}$ increased monocyte chemoattractant protein-1 (MCP-1) expression in mid-cycle but not early-cycle CL, and MCP-1 expression did not occur in large luteal cells [10]. Increased MCP-1 expression is associated with increased luteal immune cell accumulation and subsequent luteal regression [9]. A lack of MCP-1 expression may reflect a deficiency in intercellular communication after $PGF_{2\alpha}$. Thus, a number of different pathways may account for the lack of $PGF_{2\alpha}$-induced luteolysis in early CL (Day 4). This is an intriguing biological situation, in which a complete hormonal response in terms of death of the tissue is lacking in spite of sufficient hormonal receptors and clear hormonal activation of certain cellular responses. It should be noted that the current model is based almost entirely upon measurements of mRNA and will need verification in studies that evaluate $PGF_{2\alpha}$-induced changes in protein expression. Further definition of this model and unraveling of the differentiation steps that produce complete $PGF_{2\alpha}$ responsiveness will help clarify the key pathways of luteolysis and possibly the key pathways involved in elimination of a tissue.

In conclusion, we have provided evidence that $PGF_{2\alpha}$ regulates certain cellular (ascorbic acid depletion) and molecular (inhibition of mRNA for 3β-HSD and FP receptor) processes in the early- as well as mid-cycle CL. Mid-cycle CL appear to have the capacity to respond to $PGF_{2\alpha}$ by generation of intraluteal $PGF_{2\alpha}$ (via PGHS-2), induction of immunological events (e.g., MCP-1 expression), and inhibition of luteal P_4 production (possibly by inhibition of StAR). These capabilities are clearly diminished in early CL and thus might help explain the inability of early CL to undergo regression following $PGF_{2\alpha}$ treatment.

ACKNOWLEDGMENTS

The authors thank Jerry Gunther, Valerie Schutzkus, and Josie Lewandowski for their technical assistance. We also thank Rhone Merieux Inc. for providing GnRH (Cystorelin) and Pharmacia-Upjohn Co. for providing $PGF_{2\alpha}$ (Lutalyse).

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