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Prostaglandin $F_{2\alpha}$ Receptors in the Early Bovine Corpus Luteum[1]

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ABSTRACT

Since the early CL (≤4 days after ovulation) does not regress after injection of $PGF_{2\alpha}$, this study was designed to determine whether number or affinity of $PGF_{2\alpha}$ receptors was lower in the early as compared with the midstage CL. Heifers were randomly assigned to have ovaries removed on Day 2, 4, 6, or 10 (n = 4 heifers per day; Day 0 = day of ovulation). Plasma progesterone concentrations and the weight and size of the CL increased from Day 2 to 6, indicating normal CL development. Plasma membranes from individual CL were evaluated for $PGF_{2\alpha}$ receptor concentration and affinity by Scatchard analysis. CL from each of the 4 days of the estrous cycle were not different with respect to $PGF_{2\alpha}$ receptor concentration (number per microgram of plasma membrane protein) and affinity. To examine tissue specificity, $PGF_{2\alpha}$ binding was evaluated in 12 organs or tissues. High-affinity $PGF_{2\alpha}$ receptors were found in the CL and adrenal medulla but not in granulosa cells or other tissues. In conclusion, a single class of high-affinity $PGF_{2\alpha}$ receptors was present within the bovine CL by 2 days after ovulation; therefore the reported lack of responsiveness to $PGF_{2\alpha}$ in the early CL was not attributable to a deficiency of high-affinity $PGF_{2\alpha}$ receptors.

INTRODUCTION

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is a potent luteolytic agent in cattle and has been used extensively to synchronize estrus [1–8]. One of the limitations on the use of $PGF_{2\alpha}$ for estrous synchronization is its apparent ineffectiveness during the first few days of the estrous cycle [2, 7, 8]. Exogenous $PGF_{2\alpha}$ causes regression of the bovine CL only between Day 5 and Day 16 after estrus [2, 7, 8]. This lack of $PGF_{2\alpha}$ responsiveness could be due to a deficiency in number or affinity of $PGF_{2\alpha}$ receptors in the early CL.

Binding of $PGF_{2\alpha}$ to membranes of the early bovine CL has been reported [9, 10]; however, the number and affinity of these receptors have not been previously quantified. In swine there are increased numbers of high-affinity luteal receptors for $PGF_{2\alpha}$ on approximately the same day (Day 13 after estrus) that the CL becomes responsive to a single injection of $PGF_{2\alpha}$ [11]. The objective of the present study was to determine whether or not the lack of responsiveness of the early bovine CL to $PGF_{2\alpha}$ is attributable to alterations in the concentration or affinity of $PGF_{2\alpha}$ receptors. In addition, the distribution of $PGF_{2\alpha}$ receptors in various tissues was examined.

MATERIALS AND METHODS

Animals, Ultrasonic Imaging, and CL Collection

Sixteen nulliparous Holstein heifers (age = 2.5–3 yr; weight = 500–700 kg) were used during the months of October to December. Heifers were kept in outdoor paddocks with free access to shelter and were fed hay and concentrates.

Ultrasound examinations of ovaries were performed daily by a single operator. The ultrasound scanner was a real-time, B-mode instrument equipped with a 7.5-MHz, linear-array, intrarectal transducer (Aloka 210-DXII; Corometrics, Wallingford, CT). The day of ovulation was determined by the disappearance of a large follicle; and the diameter, cross-sectional area, and volume of the CL were determined by daily ultrasound as previously described [12]. On the day of detected ovulation, heifers were assigned randomly to day of slaughter (Days 2, 4, 6, and 10 postovulation; n = 4 heifers per day), with an animal assigned to each of the 4 days after ovulation prior to beginning the next block of animals.

Ovaries were collected within 15 min of slaughter. The CL was dissected from the surrounding ovarian tissue and placed into a 50-ml culture tube containing M199 (#M 0393 [Sigma Chemical Co., St. Louis, MO] supplemented with 20 mM HEPES [#H-3375, Sigma], 4.2 mM $NaHCO_3$, 100 IU/ml penicillin-G, and 10 μg/ml streptomycin sulfate), chilled on ice, and returned to the laboratory (~2 h). CL were frozen in 1 ml homogenization buffer (pH 7.0) containing 10 mM Tris-Cl, 1 mM $CaCl_2$-$(H_2O)_2$, 1 mM $MgCl_2$, 0.02% NaN_3, and 250 mM sucrose and stored at −80°C.

Assay of Progesterone

Blood was sampled daily, starting from Day 0 (the day of ovulation), until the day of slaughter. Samples were obtained from coccygeal vessels into a heparinized, evacuated tube and kept on ice until centrifugation. After centrifugation, the plasma was separated and stored at −20°C until assayed for progesterone.

Progesterone concentrations were estimated by RIA. A solid-phase RIA kit (Coat-A-Count Progesterone, #TKPG1;

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Diagnostic Products Co., Los Angeles, CA) with ^{125}I as the tracer was used without extraction as validated by Peter and Bosu [13]. Standard curves were constructed using progesterone-free (charcoal stripped) bovine plasma.

$PGF_{2\alpha}$ Radioreceptor Assay

On the day of receptor assays (four tissues of a block analyzed on one day), luteal tissue was thawed and homogenized in an SDT Tissumizer (2 × 10 sec at 24 000 rpm; SDT-080EN probes, Takmar Co., Cincinnati, OH) followed by a glass Dounce homogenizer. Homogenized tissues were centrifuged three times at 3000 × *g* for 5 min to remove tissue debris, and the supernatant was recentrifuged at 30 000 × *g* for 30 min to obtain the plasma membrane pellet. These procedures were performed at 4°C. The pellets were resuspended in homogenization buffer and the protein content was determined by the Bio-Rad microtiter plate method (Bio-Rad Protein Assay, #500–0006; Bio-Rad Labs., Richmond, CA) using BSA as the standard.

The $PGF_{2\alpha}$ radioreceptor assays were performed by a modification of the methods of Wiepz et al. [14] in 96-well, round-bottom polystyrene assay plates (Corning #25881–96; Dow Corning Co., Midland, MI). Samples of plasma membrane were centrifuged at 30 000 × *g* for 30 min; the resulting pellet was resuspended in assay buffer (pH 6.0) containing 10 mM Tris-HCl, 10 mM $CaCl_2$-$(H_2O)_2$, 1 mM $MgCl_2$, 0.02% NaN_3, and 0.01% gelatin to a concentration of 25 or 50 μg plasma membrane protein/50 μl. The individual CL were assayed with 25 μg of plasma membranes. To assess the quantitative nature of the Scatchard analyses, a pool of luteal plasma membranes was produced on each day after ovulation by combining 600 μg of plasma membrane protein from each CL. The concentration and affinity of $PGF_{2\alpha}$ receptors were determined on these pools of plasma membrane for each day at both 25 and 50 μg of plasma membrane protein. The plasma membrane was incubated with 25 μl of ^3H-$PGF_{2\alpha}$ (0.1 pmol of 5,6,8,9,11,12,14,15-^3H-N-$PGF_{2\alpha}$; NET 433 from New England Nuclear, Boston, MA, specific activity = 168.0 Ci/mmol) and 25 μl of varying concentrations of nonradioactive $PGF_{2\alpha}$ (0–0.1 nmol of the free acid of $PGF_{2\alpha}$; #16010 from Cayman Chemical Co., Ann Arbor, MI) in a final volume of 100 μl in each well. Plates were incubated for 2 h at room temperature on a vibrating titer plate shaker. A cell harvester (Mini-Mash II; BioWhittaker, Walkersville, MD) was used to separate bound and free ^3H-$PGF_{2\alpha}$ by filtration through glass microfiber paper (#34–6070–03, Whatman, Clifton, NJ) presoaked for 1 h in 0.3% polyethylenimine. The paper with the filtered plasma membrane preparation was washed ten times with 200 μl of 4°C wash buffer (10 mM Tris-HCl, 1 mM $CaCl_2$-$[H_2O]_2$, 1 mM $MgCl_2$, and 0.02% NaN_3, pH 6.0). Radioactivity of each sample was measured in 4 ml of Ready Protein$^+$ scintillation fluid (#158727; Beckman Instr., Palo Alto, CA) on a Packard Minaxi Tri-Carb 4000 (Packard Instrument Company, Inc., Downers Grove, IL) liquid scintillation counter.

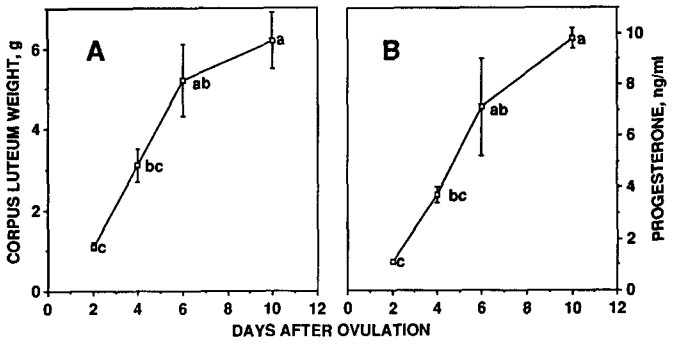

FIG. 1. Weight of CL (A) and circulating concentrations of progesterone (B) at 2, 4, 6, and 10 days after ovulation (n = 4 heifers per day; mean ± SEM). Within each endpoint, days with different subscripts are different ($p < 0.05$).

The nonradioactive $PGF_{2\alpha}$ displacement method and Scatchard analysis were used to calculate the number (B_{max}; × intercept) and affinity (K_d; −1/slope) of $PGF_{2\alpha}$ receptors. These analyses were performed on individual CL and on the pool of plasma membrane for each day.

Tissue Specificity of $PGF_{2\alpha}$ Receptors

To evaluate binding to granulosa cells, large follicles (>10 mm diameter) were aspirated from ovaries removed from heifers and cows at the time of slaughter. CL were also removed from these ovaries. Three cows were slaughtered for evaluation of $PGF_{2\alpha}$ binding to non-ovarian tissues, and a variety of tissues were removed, including muscle, brain, mammary gland, lung, liver, adrenal cortex, adrenal medulla, kidney, endometrium, and myometrium. Tissues were immediately frozen and plasma membrane preparations were isolated as described above for luteal tissue. Receptors for $PGF_{2\alpha}$ were evaluated on 200 μg plasma membrane protein by use of specific displacement of ^3H-$PGF_{2\alpha}$ by nonradioactive $PGF_{2\alpha}$ followed by Scatchard analysis.

Statistics

Data for luteal weight, plasma progesterone concentration, and $PGF_{2\alpha}$ receptor concentration and affinity were subjected to least squares analysis of variance using the General Linear Models procedure of the Statistical Analysis System (SAS, Cary, NC). Differences between means were determined by Bonferoni's multiple comparison test. The correlation of progesterone concentration vs. CL weight, diameter, cross-sectional area, and volume was determined by a simple linear regression model of SAS.

RESULTS

Correlation of CL Size and Plasma Progesterone Concentration

The CL weight and plasma progesterone concentration increased from Days 2 to 6 (Fig. 1). The plasma progester-

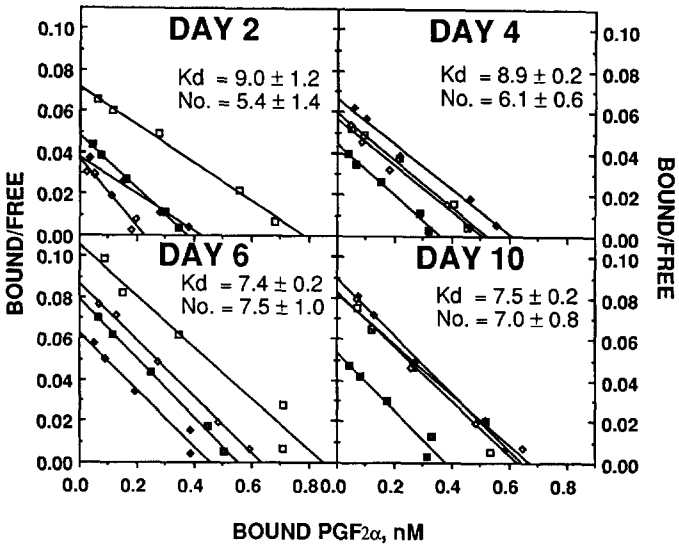

FIG. 2. Scatchard analyses of the binding of ^{3}H-$PGF_{2\alpha}$ to receptors of individual CL from 2, 4, 6, and 10 days after ovulation. Each line represents the data from luteal tissue of a different heifer. The mean ± SEM for receptor affinity (K_d, nM) and for receptor concentration (No.; 10^8/μg protein) is listed for each day. There were no differences in receptor concentration ($p = 0.22$) or receptor affinity ($p = 0.49$) on different days.

one concentration was highly correlated with luteal weight ($r = 0.92; p < 0.001$), diameter ($r = 0.77; p < 0.001$), cross-sectional area ($r = 0.73; p < 0.002$), and volume ($r = 0.79$; $p < 0.001$).

$PGF_{2\alpha}$ Receptors

The Scatchard analyses of displacement binding for individual CL are shown in Figure 2. Statistical analysis of the results with individual CL revealed no significant differences in receptor concentration (number of receptors per microgram plasma membrane protein) or affinity at different days of the estrous cycle.

Receptor concentration and affinity, as calculated from an extensive analysis of the pooled samples on Days 2, 4, 6, and 10 postovulation, were similar to the results found with individual CL (Table 1). The receptor affinity and concentration in these pooled samples were similar at the two levels of tissue, indicating that differing numbers of receptors could be accurately quantified in this Scatchard analysis.

TABLE 1. $PGF_{2\alpha}$ receptor affinity and concentration based on Scatchard analyses of pooled CL using two levels of plasma membrane protein.

Day from Ovulation	No. of CL in pool	Affinity (K_d, nM)		Concentration (10^8/μg protein)	
		25μg	50μg	25μg	50μg
Day 2	3	8.85	9.26	3.85	4.26
Day 4	4	7.37	7.39	4.51	5.17
Day 6	4	6.35	6.16	6.15	6.28
Day 10	4	6.64	6.36	5.95	6.13

TABLE 2. Specific $PGF_{2\alpha}$ binding to 200 μg plasma membrane protein from bovine tissues.

Tissue[b]	^{3}H-$PGF_{2\alpha}$ specific binding, dpm[a]			High affinity binding[c]
	Cow 834	Cow 788	Cow 1334	
Corpus luteum	18103	494	9060	Yes
Muscle	114	10	—	No
Brain	188	84	—	No
Mammary gland	0	—	227	No
Lung	187	73	223	No
Liver	81	194	203	No
Adrenal cortex	60	175	93	No
Adrenal medulla	1551	2907	2600	Yes
Kidney	91	134	162	No
Ovarian stroma	108	52	207	No
Endometrium	41	81	158	No
Myometrium	198	13	187	No

[a]Using 53,358 dpm of ^{3}H-$PGF_{2\alpha}$ (.1 pmol), 200 μg plasma membrane protein, and with or without 100 pmol nonradioactive $PGF_{2\alpha}$ for nonspecific binding. Specific binding was total binding minus nonspecific binding.
[b]The tissues were sampled from two Brown Swiss cows (#834 and #788) and one Holstein cow (#1334) at the slaughterhouse. Cow #834 and #1334 were at the midluteal phase of the cycle with an active CL; cow #788 was at the follicular phase with a regressing CL.
[c]High-affinity binding was determined by nonradioactive $PGF_{2\alpha}$ displacement and Scatchard analyses.

Tissue Specificity of $PGF_{2\alpha}$ Receptors

CL from ovaries collected at a slaughterhouse at nonselected stages of the estrous cycle had high-affinity $PGF_{2\alpha}$ receptors (K_d = 8.33 nM; 4029 dpm bound of 40 000 dpm added), whereas no high-affinity $PGF_{2\alpha}$ receptors were found on granulosa cells (no high-affinity binding; 112 dpm bound of 40 000 dpm added). This lack of $PGF_{2\alpha}$ receptors was not due to loss of all plasma membrane receptors, since FSH and LH receptors were abundant in this pool of granulosa cell plasma membrane (unpublished results). A variety of other tissues bound small amounts of ^{3}H-$PGF_{2\alpha}$, but high amounts of specific binding and high-affinity receptors were found only in CL and adrenal medulla (Table 2). The affinity of $PGF_{2\alpha}$ receptors was similar in the adrenal medulla and CL; however, the concentration of receptors was about four times greater in the active CL (Table 3).

DISCUSSION

The central hypothesis of these studies was that the lack of responsiveness to $PGF_{2\alpha}$ in the early bovine CL is due to a deficiency of high-affinity receptors for $PGF_{2\alpha}$. Displacement curves and Scatchard analyses of individual CL

TABLE 3. $PGF_{2\alpha}$ receptor affinity and concentration based on Scatchard analyses of plasma membranes (200 μg protein) from adrenal medulla and CL.

Cow ID	Reproductive Stage	Affinity (K_d, nM)		Concentration (10^8/μg protein)	
		CL	Adrenal Medulla	CL	Adrenal Medulla
834	Midluteal	5.34	10.30	8.45	1.02
788	Follicular	30.88	10.14	0.87	2.03
1334	Midluteal	7.45	13.04	9.07	2.64

and pools of CL from the same day showed no difference between developing (Days 2 and 4) and active (Days 6 and 10) CL in either affinity or concentration of $PGF_{2\alpha}$ receptors. One would expect absolute numbers of luteal $PGF_{2\alpha}$ receptors to increase with increasing size of the CL; however, concentration of receptors was not increased. It seems clear that increased numbers of $PGF_{2\alpha}$ receptors are not located on all tissues of the body, since we could not detect high-affinity $PGF_{2\alpha}$ receptors on granulosa cells or on a number of other tissues. From the results of this study it is not possible to determine the luteal cell type that contained receptors for $PGF_{2\alpha}$. The use of homogenized tissue circumvented problems of cell dissociation and receptor internalization but made it impossible to determine whether or not there were changes in receptor concentrations on specific cell types only. In sheep and pigs the high-affinity receptors for $PGF_{2\alpha}$ appear to be specifically located on the large luteal cells [11, 15]. Large luteal cells primarily arise from granulosa cells in the early bovine CL [16]. The lack of $PGF_{2\alpha}$ receptors on granulosa cells suggests that during the luteinization process (prior to 2 days after ovulation), the cells that differentiate into the large luteal cells begin to express the gene for the $PGF_{2\alpha}$ receptor.

Plasma membranes of CL from cattle and other species contain high-affinity $PGF_{2\alpha}$ receptors. In the present study we found only a single class of high-affinity $PGF_{2\alpha}$ receptor (K_d = 6–9 nM). This finding agrees with those of Powell et al. [17], Kimball and Lauderdale [18], and Mattioli et al. [19], who reported a single receptor population on the bovine CL (K_d of 50, 21, and 10 nM). Other investigators [20–22] reported two classes of $PGF_{2\alpha}$ receptors with high and low affinities (K_d of 5.1, 18; 1.6, 24; 1.4, 63 nM). The differences in affinity for the $PGF_{2\alpha}$ receptor in different studies may be due to different methods for receptor assays or plasma membrane preparation.

In the rat, the greatest specific binding for $PGF_{2\alpha}$ was found in the ovary, but specific binding was also present in oviduct, uterus, breast, pancreas, and heart [22]. In the present study, we found specific binding of ^{3}H-$PGF_{2\alpha}$ in a variety of bovine tissues also. However, Scatchard analysis of this binding indicated that high-affinity $PGF_{2\alpha}$ binding was present only in the CL and adrenal medulla. It is not clear whether or not differences in protein stability in different tissues during homogenization and freezing may account for differences in $PGF_{2\alpha}$ binding. The finding that large numbers of high-affinity $PGF_{2\alpha}$ receptors were present in the adrenal medulla was unexpected and to our knowledge has not been reported previously. It raises the possibility that $PGF_{2\alpha}$ may be involved in synthesis or secretion of catecholamines.

Recently the complete sequence of the mRNA for the mouse [23], human [24] and bovine [25] $PGF_{2\alpha}$ receptor has been reported. This receptor is part of the family of receptors that have seven transmembrane domains and are coupled to GTP-binding proteins. Consistent with our binding results, Northern analysis demonstrated that the mRNA for the $PGF_{2\alpha}$ receptor was greatest in the mouse CL [23]. In contrast, the mRNA for the $PGF_{2\alpha}$ receptor was found to be high in the mouse kidney and uterus [23], tissues in which lower amounts of binding were detected in our study of $PGF_{2\alpha}$ receptor binding. Whether this discrepancy is due to differences in species, protein/mRNA stability, or assay conditions cannot be ascertained.

The present results, while demonstrating that lack of responsiveness to $PGF_{2\alpha}$ in the early bovine CL was not attributable to a deficiency of high-affinity $PGF_{2\alpha}$ receptors, do not allow resolution of this apparent enigma. The acute in vitro effects of $PGF_{2\alpha}$ involve activation of phospholipase C and increased free intracellular calcium ion concentration [26, 27]. Coupling of the $PGF_{2\alpha}$ receptor to these intracellular effectors may be lacking in the early CL. In preliminary studies we found that approximately 20% of luteal cells from early or mid-cycle CL show increased free intracellular calcium concentrations after treatment with 100 nM $PGF_{2\alpha}$ (unpublished results). This result is consistent with a coupling of $PGF_{2\alpha}$ receptors in the early CL to the free calcium second messenger pathway; however, accurate quantification of this coupling will require more extensive investigations. Other possible explanations for the absence of regression of the early CL after injection of $PGF_{2\alpha}$ could be incomplete vascularization [28] or incomplete differentiation of degenerative mechanisms in luteal cells. Multiple i.m. injections of $PGF_{2\alpha}$ during the early luteal phase will cause regression of the CL of some animals ([5]; J.R. Chenault, unpublished results), indicating the capacity for regression in the early CL of some animals. Understanding the cellular mechanisms involved in diminished responsiveness to $PGF_{2\alpha}$ in the early CL may help in developing efficient methods for regressing the early CL.

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