Steady-state concentrations of mRNA encoding two inhibitors of protein kinase C in ovine luteal tissue

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Prostaglandin F_{2α} (PGF_{2α}) decreases secretion of progesterone from the corpus luteum in domestic ruminants. However, it is less effective during the early part of the oestrous cycle (Louis *et al.*, 1973) and at the time of maternal recognition of pregnancy (Silvia and Niswender, 1984; Lacroix and Kann, 1986). Decreased luteal responsiveness may be due to failure of PGF_{2α} to activate fully its normal second messenger system, protein kinase C (PKC). Alternatively, increased resistance of the corpus luteum to PGF_{2α} might be attributable to greater concentrations of recently identified biological inhibitors of PKC. These possibilities were addressed by measuring steady-state concentrations of mRNA encoding PGF_{2α} receptor and two inhibitors of PKC, protein kinase C inhibitor-1 (PKCI-1) and kinase C inhibitor protein-1 (KCIP-1, brain 14-3-3 protein), in corpora lutea collected from ewes on days 4, 10 and 15 of the oestrous cycle (*n* = 5 per day) and day 15 of pregnancy (*n* = 7). There were no differences in mean concentrations of mRNA encoding PGF_{2α} receptor among the groups. However, concentrations of mRNA encoding both inhibitors of PKC were higher (*P* < 0.01) on day 4 of the oestrous cycle compared with the other groups. Treatment of ewes with a luteolytic dose of PGF_{2α}, which activates PKC, did not change concentrations of mRNA encoding either PKCI-1 or KCIP-1 up to 24 h later. Luteal expression of mRNA encoding the PKC inhibitors and PGF_{2α} receptor was also examined in ewes treated with oestradiol *in vivo* for 16 h in the midluteal phase. High concentrations of oestradiol in serum (20 and 70 pg ml⁻¹) did not influence quantities of any of the mRNAs examined. Therefore, an increase in PKC inhibitors may be involved in resistance of the corpus luteum to PGF_{2α} during the early part of the oestrous cycle but does not appear to mediate the increased resistance of the corpus luteum to PGF_{2α} during maternal recognition of pregnancy. Neither PGF_{2α} nor oestradiol affected steady-state concentrations of mRNAs encoding PKCI-1 or KCIP-1.

Introduction

In domestic ruminants, pulsatile release of PGF_{2α} from the uterus induces luteal regression (reviewed in Knickerbocker *et al.*, 1988). PGF_{2α} binds to high-affinity receptors on large luteal cells resulting in activation of PKC and an influx of Ca²⁺ into the cells (Wiltbank *et al.*, 1991). Activation of PKC results in decreased progesterone production in both steroidogenic luteal cell types (Wiltbank *et al.*, 1989) through multiple mechanisms, including reduced transport of cholesterol to cytochrome P450 side-chain cleavage (P450_{sc}); Wiltbank *et al.*, 1993) and decreased concentrations of mRNAs encoding

proteins important in the steroidogenic pathway (McGuire *et al.*, 1994; Juengel *et al.*, 1995).

In cattle, administration of PGF_{2α} during the early luteal phase of the reproductive cycle does not result in luteal regression (Louis *et al.*, 1973). In addition, corpora lutea of pregnant ewes are more resistant to PGF_{2α} than those of non-pregnant ewes during maternal recognition of pregnancy (Silvia and Niswender, 1984; Lacroix and Kann, 1986). Differences in luteal responsiveness to PGF_{2α} could be due to fewer receptors for PGF_{2α} or an inability of the receptor to fully activate its second messenger systems. Recently, Wiltbank *et al.* (1995) demonstrated the presence of equal concentrations of PGF_{2α} receptors in bovine corpora lutea on days 2 and 10 of the oestrous cycle and Juengel *et al.* (1996) reported that mRNA encoding the PGF_{2α} receptor did not change between days 3 and 15 of the oestrous cycle in the ovine corpus luteum. Therefore, numbers of PGF_{2α} receptors do not appear to limit

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the responsiveness of the corpus luteum to $\text{PGF}_{2\alpha}$ during the early phase of the oestrous cycle or at the time of maternal recognition of pregnancy (Wiepz *et al.*, 1992).

Ovine luteal cells treated with conceptual proteins show an increased resistance to pharmacological activation of PKC (Wiltbank *et al.*, 1992), suggesting that the reduced responsiveness of corpora lutea to $\text{PGF}_{2\alpha}$ during maternal recognition of pregnancy occurs after binding of $\text{PGF}_{2\alpha}$ to its receptor. Direct inhibition of PKC activity is an alternative explanation of the reduced responsiveness of the corpus luteum to $\text{PGF}_{2\alpha}$. Naturally occurring proteins that inhibit PKC activity have been detected in several tissues, including the ovary (Melner, 1996). PKCI-1 is a 13 690 Da zinc-binding protein that inhibits PKC even in the presence of high concentrations of calcium and phospholipid which are normal activators of the enzyme. The inhibitor is not a phosphatase nor does it prevent PKC activation by interacting with co-factors that are essential for activity (Pearson *et al.*, 1990). PKCI-1 belongs to the HIT family of proteins, designated as such due to a conserved histidine triad in the carboxy terminal domain that forms the zinc chelation site. Although the cellular functions of PKCI-1 are not well understood, recent data indicate that members of the HIT family of genes display aberrant transcripts in a high percentage of digestive tract cancers (Ohta *et al.*, 1996). However, PKCI-1 was recently identified as the single positive clone out of 300 000 colonies in a yeast two-hybrid screen with the regulatory region of human $\text{PKC}\beta$ as bait (Lima *et al.*, 1996) which supports the theory that it acts as an inhibitor of PKC. KCIP-1 is an unrelated PKC inhibitor which is a member of a family of proteins (brain 14-3-3 proteins) that inhibit PKC and have molecular masses of 29–33 kDa. The mechanism by which KCIP-1 inhibits PKC is not known; however, it is not due to competition for co-factors required for PKC activation (Toker *et al.*, 1992). It is possible that different concentrations of one or both of these proteins in corpora lutea could explain differences in resistance to $\text{PGF}_{2\alpha}$. Therefore, the objectives of the present study were: (1) to examine luteal concentrations of mRNAs encoding $\text{PGF}_{2\alpha}$ receptor, PKCI-1 and KCIP-1 under conditions in which resistance of corpora lutea to $\text{PGF}_{2\alpha}$ is known to vary; and (2) to determine the effects of $\text{PGF}_{2\alpha}$ and oestradiol, two hormones that are known to increase the luteolytic efficacy of $\text{PGF}_{2\alpha}$ (Gengenbach *et al.*, 1977; Schramm *et al.*, 1983), on concentrations of these mRNAs.

Materials and Methods

Western Range ewes (3–6 years old; 40–70 kg) exhibiting normal oestrous cycles (18 ± 1 days) were used in all experiments. Animals were housed in dry lot pens and fed 1.8–2.3 kg of alfalfa hay per day and had free access to water. Ewes were observed for oestrus twice a day in the presence of vasectomized rams. Corpora lutea were collected surgically via midventral laparotomy. All experiments were approved by the Colorado State University or the University of Wyoming Animal Care and Use Committee.

Experiment 1

Corpora lutea were collected from nonpregnant ewes on days 4, 10 and 15 of the oestrous cycle ($n = 5$ per day) and

from pregnant ewes on day 15 of pregnancy ($n = 7$) to test the hypothesis that resistance of the corpus luteum to $\text{PGF}_{2\alpha}$ may be due to increased concentrations of PKCI-1 and KCIP-1. All pregnant ewes had viable embryos at the time of luteal tissue collection. Corpora lutea were decapsulated, weighed, frozen in liquid nitrogen and stored at -70°C until isolation of polyadenylated (poly(A)^+) RNA. Blood samples were obtained from each ewe just before tissue collection for quantification of progesterone in a single radioimmunoassay (Niswender, 1973). The coefficient of variation (CV) was 8% and the limit of sensitivity was 57 pg ml^{-1} .

Experiment 2

Steady-state concentrations of mRNAs encoding the PKC inhibitors varied during the oestrous cycle. Therefore, the objective of Expt 2 was to determine whether $\text{PGF}_{2\alpha}$ regulated expression of these mRNAs. On days 11–12 of the oestrous cycle ewes were injected i.v. with 25 mg $\text{PGF}_{2\alpha}$ (Lutalyse, Upjohn Co., Kalamazoo, MI) followed 2 h later by an i.m. injection of 10 mg $\text{PGF}_{2\alpha}$. Corpora lutea were collected from ewes 4, 12 or 24 h after the second injection ($n = 4$ or 5 per time point). Corpora lutea were also collected from untreated ewes on day 11 or 12 of the oestrous cycle and from ewes 24 h after administration of saline on day 11 or 12 of the oestrous cycle ($n = 4$ per group). Corpora lutea were processed as described for Expt 1. Jugular blood samples were obtained at the time of tissue collection and progesterone concentration was determined in sera in a single assay (CV = 14%, limit of sensitivity = 90 pg ml^{-1}).

Experiment 3

The objective of Expt 3 was to evaluate the influence of increased concentrations of oestradiol in serum on the expression of mRNAs encoding PKC inhibitors and $\text{PGF}_{2\alpha}$ receptors. Ewes received one ($n = 6$) or five ($n = 5$) oestradiol implants s.c. on day 10 of the oestrous cycle and corpora lutea were collected 16 h after implantation (Turzillo *et al.*, 1995). Corpora lutea were also collected from untreated control ewes ($n = 6$) on day 10 of the oestrous cycle. Blood samples were obtained from each ewe at 2 h intervals beginning 16 h prior to tissue collection. The oestradiol concentration in all resulting samples was determined in a single radioimmunoassay (CV = 6%, sensitivity = 0.44 pg ml^{-1} ; Thompson *et al.*, 1978) and the progesterone concentration in the samples obtained just prior to tissue collection was determined in a single assay (CV = 13%, sensitivity = 30 pg ml^{-1}).

Quantification of mRNA encoding $\text{PGF}_{2\alpha}$ receptor, PKCI-1 and KCIP-1

Unless otherwise specified, all materials used in these experiment were purchased from Sigma Chemical Co. (St Louis, MO) or Fisher Scientific (Denver, CO). Concentrations of $\text{PGF}_{2\alpha}$ receptor mRNA were quantitated as described by Juengel *et al.* (1996) using a cDNA encoding a portion of the ovine $\text{PGF}_{2\alpha}$ receptor. Slot blot analyses were performed using [^{32}P]-deoxycytidine-5'-triphosphate (dCTP)-labelled cDNA (specific

activity $0.3\text{--}1.0 \times 10^9$ disintegrations per minute (d.p.m.) μg^{-1} DNA; Feinberg and Volgelstein, 1983). Intra-assay CV averaged 12% and interassay CV was 14%. All standard curves were linear ($r = 0.99$). The sensitivity of detection of $\text{PGF}_{2\alpha}$ receptor mRNA averaged $0.44 \text{ fmol poly(A)}^+ \text{ RNA } \mu\text{g}^{-1}$.

A 360 base pair (bp) cDNA encoding bovine PKCI-1 (Melner *et al.*, 1993) was used to quantitate mRNA encoding PKCI-1 in ovine luteal tissue. Specificity of this cDNA was determined with northern analysis (Sambrook *et al.*, 1989). Poly(A)⁺ RNA was isolated from various ovine tissues by binding to oligo(deoxythymidine)-cellulose (oligo(dT)-cellulose, Collaborative Biomedical Products, Bedford, MA) (Badley *et al.*, 1988; Guy *et al.*, 1995). The concentration of RNA was quantified by absorption at 260 nm. Samples of poly(A)⁺ RNA (5 μg) were separated on a 1.5% agarose-formaldehyde-3[N-morpholinopropanesulfonic acid gel, transferred to a nylon filter (Hybond, Amersham Corp., Arlington Heights, IL) by capillary action, and cross-linked to filters by ultraviolet light (Stratalinker, La Jolla, CA). The filter was prehybridized overnight at 42°C in hybridization buffer (five-strength salt sodium citrate (SSC) (750 mmol sodium chloride l^{-1} and 75 mmol sodium citrate l^{-1}), 0.1% (w/v) Ficoll 400, 0.1% (w/v) polyvinylpyrrolidone, 0.1% (w/v) BSA (US Biochemicals, Cleveland, OH), 0.1% (w/v) SDS, 50% (v/v) deionized formamide (Gibco BRL, Gaithersburg, MD), 50 mmol $\text{NaPO}_4 \text{ l}^{-1}$, and 100 $\mu\text{g ml}^{-1}$ heat-denatured salmon sperm DNA). The filter was then incubated in hybridization buffer containing 2×10^6 counts per minute (c.p.m.) ml^{-1} PKCI-1 [³²P]cDNA (generated using the random primer method; specific activity $0.3\text{--}1.0 \times 10^9$ d.p.m. μg^{-1} DNA) at 42°C for 24 h. After hybridization, the filter was washed four times in double-strength SSC and 0.1% SDS at room temperature for 10 min and was then washed twice in 0.1 strength SSC and 0.1% SDS at 65°C for 30 min. Filters were then exposed to Kodak X-AR film (Eastman Kodak, Rochester, NY) with intensifying screens for 0.5–3 days at -70°C . The concentration of mRNA encoding PKCI-1 in individual luteal samples was determined by slot blot analysis. Poly(A)⁺ RNA was isolated from corpora lutea as described for the northern blot analysis. Duplicate aliquots of 300 ng poly(A)⁺ RNA from individual luteal samples were applied to nylon filters with a slot blot apparatus. Various quantities (5, 10, 30, 50, 100, 300 and 500 ng) of appropriate sense mRNA generated with SP6 polymerase and positive control RNA (100, 300 and 500 ng of pooled luteal poly(A)⁺ RNA) were also included on each filter. Conditions for prehybridization, hybridization and washing of filters were as described for the northern analysis. Comparison of hybridization of PKCI-1 [³²P]cDNA to samples and standard, as measured by densitometric scanning (Hoefer Scientific Instruments, San Francisco, CA) of autoradiographs, was used to calculate the concentration of mRNA encoding PKCI-1 in individual samples. All standard curves for synthesized PKCI-1 mRNA were linear with high correlation between the quantity of standard added and resulting densitometric reading ($r = 0.99$). Quantities of PKCI-1 mRNA were linear ($r = 0.99$) in 100–500 ng pooled luteal poly(A)⁺ RNA. Intra- and inter-assay CVs were less than 13% and the limit of detection averaged $0.15 \text{ fmol poly(A)}^+ \text{ RNA } \mu\text{g}^{-1}$.

cDNA was generated from luteal poly(A)⁺ RNA with a primer corresponding to bases 653–668 of the bovine KCIP-1

cDNA (Ichimura *et al.*, 1988) and reverse transcriptase to generate a cDNA encoding ovine KCIP-1. The ovine cDNA encoding KCIP-1 was amplified by PCR (40 cycles of denaturing at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 2 min; GeneAMP, Perkin Elmer Cetus, Norwalk, CT) with primers specific for bases 303–322 and 653–668 of the bovine KCIP-1 cDNA (Ichimura *et al.*, 1988). The resulting 355 bp fragment was ligated into pCRII (Invitrogen, San Diego, CA) and the sequence determined by the dideoxy procedure (Sequenase Version 2.0, US Biochemicals, Cleveland, OH; Chen and Seeburg, 1985). The insert was released from the plasmid with Eco RI and gel purified. The purified insert was used for northern and slot blot analyses after incorporation of [³²P]dCTP (111 TBq mmol^{-1} ; Amersham, Arlington Heights, IL) by the random primer method (specific activity $0.3\text{--}1.0 \times 10^9$ d.p.m. μg^{-1} DNA; Feinberg and Volgelstein, 1983).

Tissue distribution and size of ovine KCIP-1 mRNA were determined by northern analysis as described for PKCI-1. Quantities of mRNA encoding KCIP-1 in individual luteal samples were determined by slot blot analysis. Sense RNAs for KCIP-1 were synthesized by transcribing the Bam HI-linearized plasmid containing KCIP-1 with T7 polymerase to generate a standard curve. All standard curves were linear ($r = 0.99$). Quantities of KCIP-1 mRNA were linear ($r = 0.98$) in 100–500 ng of pooled luteal poly(A)⁺ RNA. Intra-assay CV averaged 16% and interassay CV was 25%. The limit of detection of KCIP-1 mRNA, based on the lowest detectable point in the standard curve, averaged $0.15 \text{ fmol poly(A)}^+ \text{ RNA } \mu\text{g}^{-1}$.

After hybridization to specific cDNA, all filters were hybridized with [³²P]deoxythymidine (d(T)) as described by Juengel *et al.* (1994) to determine whether equal quantities of poly(A)⁺ RNA were loaded on slots within each filter. Correlation between different concentrations of RNA applied to the filters and densitometric signals was high ($r = 0.97 \pm 0.01$). CV between densitometric reading of samples averaged 18% for all filters. Therefore, measurements of mRNA encoding $\text{PGF}_{2\alpha}$ receptor, PKCI-1 and KCIP-1 were not corrected for differences in RNA loading.

Statistical analysis

In ewes with multiple ovulations, the average mass of corpora lutea was used for statistical analysis. All analyses were performed using the general linear models procedures of SAS (1989). In Expt 2, there were no differences between saline-injected and non-injected controls in any parameter examined. Therefore, data from these two groups were combined prior to final statistical analysis and are referred to as controls. In Expt 3 concentrations of oestradiol in sera (average of samples collected during the last 14 h before tissue collection) were square root transformed prior to final analysis due to heterogeneity of variance. However, means $\pm$ SEM of raw data are presented. In Expts 2 and 3, values for the mRNAs ($\text{fmol poly(A)}^+ \text{ RNA } \mu\text{g}^{-1}$) were expressed as a percentage of the mean control value. Data from all experiments were tested with analysis of variance procedures and when treatment effects

Fig. 1. Autoradiograph (overnight exposure) of northern blot analysis of ovine poly(A)⁺ RNA samples (5 µg) hybridized with bovine protein kinase C inhibitor-1 cDNA. Tissue source is indicated along the top of the figure. Migration of RNA standards is shown on the right in bases.

were significant ($P < 0.05$) differences between least squares means were evaluated by least significant differences.

Results

The cDNA encoding bovine PKCI-1 hybridized to a single transcript of approximately 780 bases in all tissues examined (Fig. 1). The apparent smaller size of this transcript in luteal tissue was due to aberrant migration of RNA at the edge of the gel. The nucleotide sequence obtained from the ovine KCIP-1 cDNA was 95% identical to the corresponding region of rat brain 14-3-3 θ sequence (Watanabe *et al.*, 1994). A primary transcript of approximately 2280 bases was observed in all tissues examined following hybridization to the ovine KCIP-1 cDNA (Fig. 2). A second minor transcript of approximately 4300 bases was observed in brain and adrenal tissues.

In Expt 1, luteal mass and serum progesterone concentrations increased ($P < 0.05$) between days 4 and 10 of the oestrous cycle, as also reported by Juengel *et al.* (1995). Luteal mass was similar between days 10 and 15 of the oestrous cycle and day 15 of pregnancy. However, serum progesterone concentrations were higher ($P < 0.05$) on day 15 of pregnancy compared with days 10 or 15 of the oestrous cycle. Steady-state luteal concentrations of mRNA encoding both PKCI-1 and KCIP-1 were greater ($P < 0.01$) on day 4 of the oestrous cycle than on days 10 or 15 (Fig. 3). There was no difference in the concentration of mRNA encoding the PGF_{2α} receptor on these days (Fig. 3). Luteal concentrations of mRNAs encoding the PKC inhibitors and the PGF_{2α} receptor on day 15 of the oestrous cycle and day 15 of pregnancy were not significantly different (Fig. 3). In Expt 2, injection of a luteolytic dose of

Fig. 2. Autoradiograph (overnight exposure) of northern blot analysis of ovine poly(A)⁺ RNA samples (5 µg) hybridized with ovine protein kinase C inhibitor-1 cDNA. Tissue source is indicated along the top of the figure. Migration of RNA standards is shown on the right in bases.

Fig. 3. Ovine luteal concentrations of (■) protein kinase C inhibitor-1, (▨) kinase C inhibitor protein-1 and (□) prostaglandin F_{2α} receptor mRNA on days 4, 10 and 15 of the oestrous cycle and day 15 of pregnancy. For each class of mRNA, differences between time periods are indicated by different letters ($P < 0.01$).

PGF_{2α} resulted in a decrease in both serum progesterone concentration and luteal mass (Juengel *et al.*, 1996) but did not affect the concentrations of mRNA encoding PKCI-1 or KCIP-1 (Fig. 4). In Expt 3, ewes administered with one or five oestradiol implants had mean serum oestradiol concentrations that were 15.8- and 54.1-fold higher, respectively, than control ewes (Table 1). However, exposure to oestradiol for 16 h did not affect serum progesterone concentrations, luteal mass or luteal concentrations of mRNA encoding PKCI-1, KCIP-1 or PGF_{2α} receptor (Table 1).

Fig. 4. Concentrations of (■) protein kinase C inhibitor-1 and (▨) kinase C inhibitor protein-1 mRNA in corpora lutea collected from ewes 4, 12 or 24 h after injection with prostaglandin F_{2α}. There were no differences among groups.

Discussion

The cDNA encoding bovine PKCI-1 hybridized to a transcript of approximately 780 bases in all ovine tissues examined. The widespread distribution of this mRNA was similar to previous reports and was not unexpected due to the presence of PKC in a variety of tissues (Melner, 1996).

The partial cDNA encoding ovine KCIP-1 was 95% identical to the rat θ KCIP-1 isoform reported by Watanabe *et al.* (1994). The sequence also shared significant similarity (57–72%) to other isoforms of KCIP-1 (Watanabe *et al.*, 1991, 1993, 1994; Roseboom *et al.*, 1994). However, this cDNA sequence detected a single major transcript of approximately 2280 bases when used in northern blot analysis. Therefore, hybridization conditions were sufficiently stringent to prevent cross-hybridization of the cDNA to related isoforms of KCIP-1. This mRNA is similar in size to a major transcript for θ subtype of KCIP-1 observed in rat brain RNA although other transcripts were also observed in the rat. The differences in size of mRNA in the rat were most likely due to differences in polyadenylation sites (Watanabe *et al.*, 1994). The θ subtype of KCIP-1 protein is a potent PKC inhibitor (Robinson *et al.*, 1994) with an IC₅₀ value (the concentration that inhibits the response by 50%) of less than 50% of that observed for sheep brain isoform mixture.

Concentrations of mRNA encoding both inhibitors of PKC were higher on day 4 of the oestrous cycle compared with days 10 or 15. In rat ovary, expression of PKCI-1 mRNA decreases when actively growing follicles differentiate into corpora lutea (Melner, 1996). This decrease may continue until a fully differentiated corpus luteum is present. In contrast to the increased concentrations of mRNAs encoding PKC inhibitors during the early luteal phase, there were no differences between day 15 of the oestrous cycle and day 15 of pregnancy. Therefore, an increase in mRNAs encoding PKC inhibitors, and the associated increase in the corresponding proteins, may be involved in resistance of the corpus luteum to PGF_{2α} during the early part of the oestrous cycle, but does not appear to mediate the increased resistance of the corpus luteum to PGF_{2α} during

maternal recognition of pregnancy. Furthermore, the low expression of mRNA encoding PKC inhibitors during maternal recognition of pregnancy may explain why corpora lutea present at this stage respond to high doses of PGF_{2α} with decreased secretion of progesterone, but those present in the early luteal phase do not.

There were no differences in the steady-state concentrations of mRNA encoding PGF_{2α} receptor on days 4, 10 and 15 of the oestrous cycle and day 15 of pregnancy. These data are similar to the observations of Juengel *et al.* (1996). Wiepz *et al.* (1992) also reported that there were no differences in the number of PGF_{2α} receptors on days 13 and 15 of pregnancy or on day 13 of pregnancy compared with day 13 of the oestrous cycle. However, this study did detect more PGF_{2α} receptors on day 15 of pregnancy compared with day 15 of the oestrous cycle. In addition, Rueda *et al.* (1995) reported that concentrations of mRNA encoding PGF_{2α} receptor were higher on day 16 of pregnancy when compared with day 16 of the oestrous cycle. The discrepancies between these studies may be due to the negative effects of PGF_{2α} on its receptor mRNA (Graves *et al.*, 1995; Juengel *et al.*, 1996) and the timing of luteolysis in ewes which is initiated around day 15 of the oestrous cycle.

Multiple injections of PGF_{2α} are more effective in inducing luteolysis than a single injection of PGF_{2α} (Schramm *et al.*, 1983). The effect of treatment with PGF_{2α} on the concentrations of mRNA encoding PKC inhibitors was examined in the present study. Injection of PGF_{2α} did not affect the concentrations of mRNA encoding either PKC-1 or KCIP-1. Juengel *et al.* (1996) reported that concentrations of mRNA encoding PGF_{2α} receptor decreased to 20% of the concentration in controls 12 h after PGF_{2α} injection. Therefore, neither a decrease in PKC inhibitors nor an increase in PGF_{2α} receptor mRNA appears to be the cause of the increased sensitivity of the corpus luteum to PGF_{2α} after treatment with PGF_{2α}.

Treatment with oestradiol increases the luteolytic efficacy of PGF_{2α} (Gengenbach *et al.*, 1977) and also increases the number of PGF_{2α} receptors in luteal tissue (Bussmann, 1989). In hysterectomized ewes, treatment with a combination of oestradiol and PGF_{2α} reduces the concentration of PGF_{2α} needed to induce luteolysis (Gengenbach *et al.*, 1977). Therefore, oestradiol appears to increase the responsiveness of the corpus luteum to PGF_{2α} directly. The effect of treatment with oestradiol on concentrations of mRNA encoding PKCI-1, KCIP-1 and PGF_{2α} receptor was examined in the present study. Treatment of ewes with high doses of oestradiol for 16 h did not change luteal mass, serum progesterone concentrations or concentrations of mRNAs encoding PGF_{2α} receptor, PKCI-1 or KCIP-1. In previous studies, injection of oestradiol was found to decrease serum progesterone concentrations and luteal mass (Stormshak *et al.*, 1969; Hawk and Bolt, 1970; Hixon *et al.*, 1975). The absence of a similar response to oestradiol in corpora lutea in the present experiment may be due to the shorter exposure time of the ewes to oestradiol. Therefore, assuming that concentrations of mRNAs and their associated proteins are tightly correlated, as has been shown for the PGF_{2α} receptor (Sakamoto *et al.*, 1994), the increased efficacy of PGF_{2α} after treatment with oestradiol does not appear to be mediated through changes in PGF_{2α} receptor, PKCI-1 or KCIP-1 concentrations. Previous studies have shown that large luteal cells in ewes contain receptors for both oestradiol (Glass *et al.*, 1984)

Table 1. Serum progesterone and serum oestradiol concentrations, luteal mass and luteal concentrations of mRNAs encoding protein kinase C inhibitor-1, kinase C inhibitor protein-1 and prostaglandin $F_{2\alpha}$ receptor during the midluteal phase in ewes 16 h after treatment with oestradiol implants

	Number of oestradiol implants		
	0	1	5
Progesterone (ng ml ⁻¹)	1.8 ± 0.3	1.6 ± 0.2	1.8 ± 0.4
Oestradiol (pg ml ⁻¹)	1.3 ± 0.7 ^a	20.5 ± 6.6 ^b	70.3 ± 7.4 ^c
Luteal mass (mg)	597.0 ± 59.0	554.0 ± 20.0	543.0 ± 54.0
Protein kinase C inhibitor-1 mRNA*	100.0 ± 16.0	95.0 ± 9.0	111.0 ± 9.0
Kinase C inhibitor protein-1 mRNA*	100.0 ± 13.0	88.0 ± 8.0	93.0 ± 9.0
Prostaglandin $F_{2\alpha}$ receptor mRNA*	100.0 ± 17.0	92.0 ± 13.0	103.0 ± 20.0

Values are means ± SEM.

^{abc}Within each parameter, values with different superscripts are significantly different ($P < 0.001$).

*Percentage of corresponding values in ewes not treated with oestradiol implants.

and PGF_{2α} (Fitz *et al.*, 1982) and luteal PGF_{2α} receptors in rats are upregulated by oestradiol (Bussmann, 1989). Therefore, the absence of an effect of oestradiol on mRNA encoding PGF_{2α} receptor is surprising. Differences between the present results and those in rats may be attributed to differences between species, differences in oestradiol concentration, or different endpoint measurements (mRNA versus binding of PGF_{2α}).

In conclusion, the increased resistance of the corpus luteum to PGF_{2α} during the early part of the oestrous cycle may be due to increased concentrations of mRNA encoding PKCI-1 and KCIP, and the associated increase in the corresponding proteins. However, increased concentrations of mRNAs encoding PKCI-1 or KCIP do not appear to mediate increased resistance of the corpus luteum during maternal recognition of pregnancy. In addition, neither PGF_{2α} nor oestradiol, hormones that are known to increase the luteolytic efficacy of PGF_{2α}, affected concentrations of mRNA encoding PKCI-1 or KCIP-1. In addition, oestradiol did not increase luteal expression of PGF_{2α} receptor mRNA.

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