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Steady-state concentrations of mRNA encoding the receptor for luteinizing hormone during the estrous cycle and following prostaglandin $F_{2\alpha}$ treatment of ewes

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A partial cDNA was used to measure steady-state concentrations of mRNA encoding the receptor for luteinizing hormone (LH) in ovine corpora lutea. In experiment 1, luteal tissue and purified preparations of small and large steroidogenic luteal cells ($n = 4$ per day) were obtained on days 3 (tissue only), 6, 9, 12 and 15 of the estrous cycle (estrus = day 0). Steady-state concentrations (fmoles receptor mRNA/μg poly(A)$^+$ RNA) and total quantities of mRNA (fmoles/corpus luteum) encoding the receptor for LH in luteal tissue increased ($P < 0.05$) from day 3 to days 9 and 12 of the cycle; values on days 6 and 15 were intermediate. Small luteal cells contained at least four-fold greater ($P < 0.001$) concentrations of mRNA encoding the receptor for LH than large luteal cells on days 6, 9, 12 and 15 of the cycle. In experiment 2, ewes on days 11 or 12 of the cycle received an infusion of either 1 μmol prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or saline into the ovarian artery. Luteal tissue was collected 1 ($n = 6$), 4 ($n = 5$), 12 ($n = 5$) or 24 ($n = 5$) h following $PGF_{2\alpha}$ infusion, and 0 (no infusion; $n = 3$), 12 ($n = 3$) or 24 ($n = 4$) h following saline administration. Concentrations of progesterone in sera decreased ($P < 0.05$) within 12 h and remained low, whereas luteal weight and concentrations of progesterone in luteal tissue did not decrease ($P < 0.05$) until 24 h after $PGF_{2\alpha}$ treatment. Steady-state concentrations of mRNA encoding the receptor for LH were reduced ($P < 0.05$) within 4 h of $PGF_{2\alpha}$ infusion, and continued to decrease at 12 and 24 h post treatment. Calculated amounts of mRNA encoding the receptor for LH per corpus luteum were reduced ($P < 0.05$) at 12 h after the $PGF_{2\alpha}$ treatment and were 10% ($P < 0.05$) of the values in saline-treated ewes at 24 h post-treatment. The increase during the estrous cycle in steady-state concentrations of mRNA encoding the receptor for LH appears to occur prior to the previously observed increase in number of receptors for LH. Following $PGF_{2\alpha}$-induced luteal regression, concentrations of mRNA encoding LH receptor decreased prior to the previously reported decrease in LH binding. Thus, changes in the number of receptors for LH in ovine luteal tissue during luteal development and luteolysis appears to be preceded by corresponding changes in mRNA encoding this receptor.

Keywords: ovine; LH receptor; LH receptor mRNA; $PGF_{2\alpha}$; corpus luteum

Introduction

Luteinizing hormone (LH) is an important luteotropic hormone in most mammalian species. Infusions of LH maintained luteal function in hypophysectomized ewes (Kaltenbach *et al.*, 1968; Farin *et al.*, 1990) and prolonged the estrous cycle in intact ewes (Karsch *et al.*, 1971). Treatment of ewes during the luteal phase of the estrous cycle with antisera to LH decreased progesterone production (Fuller & Hansel, 1970; Reimers & Niswender, 1975). The biological effects of LH are mediated through specific, cell-surface receptors. In sheep, numbers of receptors for LH increased 40-fold during the early luteal phase of the estrous cycle, remained elevated for 8-12 days and then decreased at the time of luteal regression (Diekman *et al.*, 1978a,b). During the luteal phase of the estrous cycle, concentrations of progesterone in sera were more closely correlated with numbers of receptors for LH than with concentrations of LH in sera (Diekman *et al.*, 1978a; Niswender & Nett, 1994), suggesting that regulation of the number of receptors for LH may be a key mechanism controlling the secretion of progesterone by the corpus luteum.

The corpus luteum of many mammalian species consists of two distinct types of steroidogenic cells, frequently referred to as small and large luteal cells (reviewed by Niswender & Nett, 1994). In sheep, small and large luteal cells contained equivalent numbers of receptors for LH during the breeding season (Harrison *et al.*, 1987), although the surface area of large luteal cells is several times greater. Thus, the number of receptors per unit of membrane in large luteal cells is less than in small cells. Binding of LH to receptor in small luteal cells resulted in activation of adenylate cyclase, production of cAMP, and activation of protein kinase A leading to increased production of progesterone (Hoyer *et al.*, 1984). On the other hand, binding of LH to receptor in large luteal cells is not coupled to activation of adenylate cyclase and does not increase intracellular levels of cAMP or production of progesterone (Hoyer *et al.*, 1984). The biological responses of large and small luteal cells to LH differ and it seems likely that mechanisms which regulate the number of receptors for LH in the two cell types may also differ.

The DNA sequence encoding the receptor for LH has been determined in the rat (McFarland *et al.*, 1989), pig (Loosfelt *et al.*, 1989), human (Minegish *et al.*, 1990) and mouse (Gudermann *et al.*, 1992). In subsequent studies the numbers of receptors for LH and steady-state concentrations of mRNA encoding the receptor for LH were highly correlated during luteal development in rats (Segaloff *et al.*, 1990; Hu *et al.*, 1990; Hoffman *et al.*, 1991) and monkeys (Ravindranath *et al.*, 1992). The objectives of the present study were: (1) to quantitate steady-state levels of mRNA encoding the receptor for LH in ovine luteal tissue on specified days of the estrous cycle; (2) to determine the concentrations of mRNA encoding the receptor for LH in large and small ovine luteal cells; and (3) to measure concentrations of mRNA encoding the receptor for LH in luteal tissue following treatment with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) during the mid-luteal phase of the estrous cycle.

Results

There was specific hybridization of the cDNA probe for the ovine LH receptor to poly(A)$^+$ RNA isolated from ovine

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luteal tissue and purified luteal cells using Northern analysis (Figure 1). There were two major bands at 6.0 kilobases (kb) and 3.0 kb and two minor bands at 1.8 and 1.2 kb, as determined with RNA size markers. The 1.2 kb band was evident only following prolonged film exposure. The number and approximate sizes of mRNA transcripts are similar to those reported for the ovine LH receptor (Bacich *et al.*, 1994). No hybridization was observed with up to 10 μg of poly(A)$^+$ RNA isolated from ovine liver, adrenal, kidney, spleen or brain (data not shown and Figure 1). The sequence of the cDNA fragment of the ovine LH receptor used as probe was 93.7%, 86.8%, 86.8% and 85.4% homologous to those of the pig (Loosfelt *et al.*, 1989), human (Minegish *et al.*, 1990), rat (McFarland *et al.*, 1989) and mouse (Gudermann *et al.*, 1992) respectively, and except for two bases was identical where it overlapped with another partial clone of the ovine LH receptor (Bacich *et al.*, 1994).

Experiment 1

Luteal weights and progesterone concentrations in sera collected during the estrous cycle are shown in Table 1. Steady-state concentrations of mRNA encoding the receptor for LH (fmoles/μg poly(A)$^+$ RNA) in luteal tissue (Figure 2 and Table 1) increased ($P<0.05$) from day 3 to days 9 and 12 of the cycle; values on days 6 and 15 were intermediate. When the total amounts of mRNA encoding the receptor for LH per corpus luteum (fmoles/corpus luteum) were calculated (Table 1), values increased ($P<0.05$) over 10-fold from day 3 to day 12; by day 15, total amounts had decreased by 50% ($P<0.05$).

Steady-state concentrations of mRNA encoding the receptor for LH in small luteal cells were significantly higher ($P<0.001$) than in large luteal cells on all days of the estrous cycle examined (Figure 2). There were no differences in steady-state concentrations of mRNA encoding the receptor for LH on any day of the cycle examined in either large or small luteal cells.

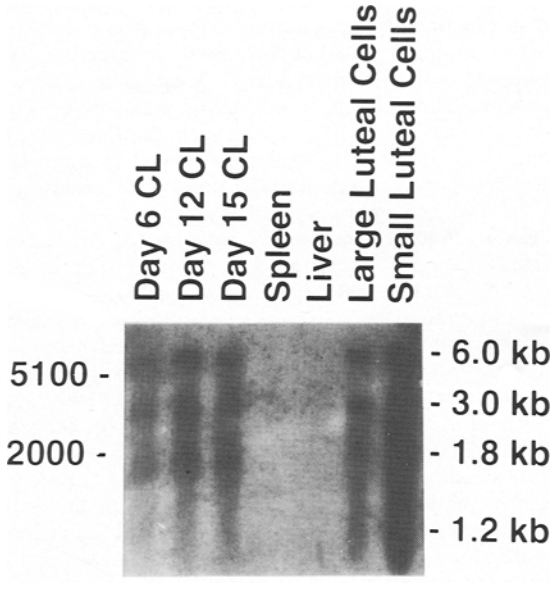

Figure 1 A representative autoradiogram following Northern blot analysis of poly(A)$^+$ RNA isolated from ovine corpus luteum, liver, spleen and purified large and small luteal cells. All lanes contained 10 μg poly(A)$^+$ RNA except for small cells, which contained 5 μg poly(A)$^+$ RNA. The autoradiogram was overexposed (17 days) to allow detection of the small, less abundant transcripts. Numbers on the left side indicate approximate sizes and locations of ribosomal RNA bands, and numbers on the right side indicate approximate sizes and locations of mRNA species that hybridized with the ovine probe for the LH receptor

Experiment 2

Concentrations of progesterone in sea (Table 2) were less ($P<0.05$) than those in saline-treated ewes (controls) at 1, 12 and 24 h after $PGF_{2\alpha}$ infusion, whereas values at 4 h post infusion were not different from controls. Luteal weights and concentrations of progesterone in luteal tissue (Table 2) were less ($P<0.05$) than control values at 24 h after $PGF_{2\alpha}$ treatment. Steady-state concentrations of mRNA encoding the receptor for LH (Table 2) were decreased ($P<0.05$) within 4 h after $PGF_{2\alpha}$ infusion and continued to decline throughout the experiment, reaching values 18% of controls at 24 h. Calculated amounts of mRNA encoding the receptor for LH per corpus luteum (Table 2) were reduced ($P<0.05$) from control values at 12 and 24 h following $PGF_{2\alpha}$ infusion.

Discussion

Specificity of the cDNA probe was demonstrated by hybridization to poly(A)$^+$ RNA isolated from ovine luteal tissue and purified luteal cells and by lack of hybridization to poly(A)$^+$ RNA isolated from several tissues which do not respond to LH. In addition, there was a high degree of homology of the probe with the LH receptor cDNA sequences from other species (Loosfelt *et al.*, 1989; McFarland *et al.*, 1989; Minegish *et al.*, 1990; Gudermann *et al.*,

Table 1 Steady-state concentrations of LH receptor mRNA (fmoles/μg poly(A)$^+$ RNA) and total LH receptor mRNA per corpus luteum (fmoles/CL), luteal weights, and concentrations of progesterone in sera on selected days of the ovine estrous cycle

Day of cycle	*fmoles μg RNA*	*fmoles CL*	*Luteal weight† (g)*	*Serum progesterone† (ng/ml)*
3	0.1 ± 0.1*[a]	0.7 ± 0.1[a]	0.15 ± 0.02[a]	0.1 ± 0.1[a]
6	0.3 ± 0.1[ab]	4.3 ± 2.2[ab]	0.33 ± 0.03[b]	0.7 ± 0.2[ab]
9	0.4 ± 0.1[b]	6.7 ± 2.0[bc]	0.36 ± 0.04[b]	1.8 ± 0.3[c]
12	0.5 ± 0.1[b]	10.4 ± 2.0[c]	0.57 ± 0.07[c]	2.6 ± 0.6[c]
15	0.4 ± 0.1[ab]	4.5 ± 1.9[ab]	0.49 ± 0.07[bc]	1.0 ± 0.4[ab]

*Mean ± SE. [a,b,c]Values within a column with different superscripts are different ($P<0.05$). †Data previously reported by Juengel *et al.* (1994)

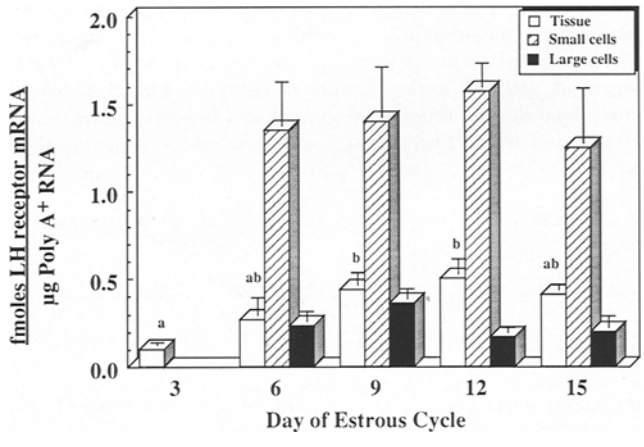

Figure 2 Mean (± SE) steady-state concentrations of mRNA encoding the receptor for LH (fmoles/μg of poly(A)$^+$ RNA) in ovine luteal tissue and small and large steroidogenic luteal cells during the estrous cycle. For luteal tissue, concentrations of mRNA encoding the receptor for LH with different letters are different ($P<0.05$). Concentrations of mRNA encoding the receptor for LH were greater ($P<0.001$) in purified preparations of small cells than large cells on all days examined. There were no differences ($P>0.05$) in concentrations of mRNA encoding the receptor for LH between days within cell types

Table 2 Steady-state concentrations of LH receptor mRNA (fmoles/μg poly(A)$^+$ RNA) and total LH receptor mRNA content (fmoles/CL), luteal weights, and concentrations of progesterone (P_4) in sera and luteal tissue at specified times after an ovarian arterial infusion of $PGF_{2\alpha}$ into ewes during the mid-luteal phase of the estrous cycle

Treatment (n)	*fmoles* μg *RNA*	*fmoles* CL	*Luteal weight* (g)	*Serum* P_4 (ng/ml)	*Luteal* P_4 (ng/mg)
Control (10)	1.1 ± 0.2*[a]	20.8 ± 3.8[a]	0.59 ± 0.02[a]	1.7 ± 0.1[a]	18.0 ± 1.9[a]
$PGF_{2\alpha}$ 1 h (6)	0.9 ± 0.2[ab]	16.6 ± 4.1[a]	0.53 ± 0.04[ab]	1.1 ± 0.1[bc]	15.2 ± 2.6[a]
$PGF_{2\alpha}$–4 h (5)	0.5 ± 0.1[bc]	11.0 ± 1.8[ab]	0.65 ± 0.06[a]	1.4 ± 0.2[ab]	14.6 ± 1.7[a]
$PGF_{2\alpha}$–12 h (5)	0.4 ± 0.1[c]	5.4 ± 1.0[b]	0.53 ± 0.07[ab]	0.8 ± 0.1[c]	12.9 ± 2.4[ab]
$PGF_{2\alpha}$–24 h (5)	0.2 ± 0.1[c]	1.9 ± 1.2[b]	0.40 ± 0.03[b]	0.2 ± 0.1[d]	6.7 ± 1.4[b]

*Mean ± SE. [a,b,c]Values within a column with different superscripts are different ($P<0.05$)

1992) and another incomplete clone of the ovine LH receptor (Bacich *et al.*, 1994). Luteal weights and concentrations of progesterone in sera in experiment 1 were similar to those reported by others (Diekman *et al.*, 1978a; Farin *et al.*, 1986) in sheep, and are indicative of normal ovine estrous cycles.

The probe used in the present study encoded the central region of the extracellular domain, whereas the probe used by Bacich *et al.* (1994) encoded the region encompassing the junction between the extracellular and transmembrane domains. There were similar sizes and numbers of mRNA transcripts encoding the receptor for LH in both studies, suggesting that both probes were able to identify the major transcripts of the receptor for LH in the ovine corpus luteum.

Concentrations of mRNA encoding the receptor for LH on day 6 of the cycle were not different from maximal values. However, numbers of receptors for LH on day 6 were still low in both ovine luteal tissue (Diekman *et al.*, 1978a) and isolated luteal cells (Harrison *et al.*, 1987), suggesting that concentrations of mRNA encoding the receptor for LH are elevated before numbers of receptors for LH are increased. Concentrations of mRNA encoding the receptor for LH were also increased during luteal development in monkeys (Ravindranath *et al.*, 1992) and superovulated rats (Hu *et al.*, 1990).

Small luteal cells contained 4–8 fold greater concentrations of mRNA encoding the receptor for LH than large luteal cells on all days of the cycle examined. Differences in concentrations of mRNA encoding the receptor for LH between small and large luteal cells may be greater when two aspects of the cell purification process are considered. First, preparations of small luteal cells are contaminated with up to 50% nonsteroidogenic cells (Fitz *et al.*, 1982); therefore, specific activity of LH receptor mRNA per small luteal cell is likely higher than reported here. Second, the preparations of large luteal cells used for the present study was contaminated (mean = 11%) with small luteal cells. Thus, a portion of the mRNA encoding the receptor for LH isolated from partially purified preparations of large luteal cells was due to contamination with small luteal cells. Based on this information it seems clear that differences in concentrations of mRNA encoding the receptor for LH in the two steroidogenic luteal cell types reported here are conservative estimates. This result was expected since the concentrations of LH receptor per unit of cell membrane is much less in large *vs* small luteal cells, although number of receptors per cell are similar (Harrison *et al.*, 1987).

In experiment 2, infusion of $PGF_{2\alpha}$ decreased concentrations of progesterone in sera and luteal tissues, indicating that luteal regression was induced by the treatment. The decreased concentrations of progesterone in sera at 1 h post infusion may have resulted from occlusion of the ovarian artery during infusion, and the fact that progesterone concentrations returned to control values at 4 h suggests that this effect was not due to a specific, long term effect of $PGF_{2\alpha}$.

Steady-state concentrations of mRNA encoding the receptor for LH were reduced within 4 h following $PGF_{2\alpha}$ treatment. The effects of $PGF_{2\alpha}$ appear to be specific, as changes in concentrations of mRNA encoding the receptor for LH were not observed in saline-infused animals. Other studies using this infusion technique have shown that luteal concentrations of mRNA encoding P450 side-chain cleavage enzyme were unchanged 24 h after $PGF_{2\alpha}$ infusion, whereas luteal concentrations of mRNA encoding 3β-hydroxysteroid dehydrogenase were reduced by $PGF_{2\alpha}$ treatment (McGuire *et al.*, 1994). Concentrations of mRNA encoding the receptor for LH were rapidly reduced by $PGF_{2\alpha}$ infusion, well before the $PGF_{2\alpha}$-induced decrease in numbers of receptors for LH reported by Diekman *et al.* (1978b). Although it would be expected that timing of onset of the effects of $PGF_{2\alpha}$ varied between the study of Diekman *et al.* (1978b) and the present study, ovarian arterial infusion of $PGF_{2\alpha}$ was used in the present study to insure precise timing of initiation of the actions of $PGF_{2\alpha}$.

Prostaglandins $F_{2\alpha}$ binds specifically to high affinity receptors localized on large luteal cells (Fitz *et al.*, 1982) resulting in activation of protein kinase C which leads to decreased progesterone secretion (Wiltbank *et al.*, 1990). Due to a lack of receptor, isolated small luteal cells do not directly respond to $PGF_{2\alpha}$ (Wiltbank *et al.*, 1990). *In vivo*, large luteal cells synthesize 80% of the progesterone secreted by the corpus luteum (Niswender *et al.*, 1985), however, induction of luteolysis with $PGF_{2\alpha}$ causes regression of both cell types in ovine corpora lutea (Braden *et al.*, 1988). In the present study, concentrations of mRNA encoding LH receptors rapidly decreased following $PGF_{2\alpha}$ induced luteal regression. However, small luteal cells, which do not possess receptors for $PGF_{2\alpha}$ appear to contain most of the mRNA encoding the receptor for LH. Thus, large luteal cells must be signalling small luteal cells to down-regulation LH receptor mRNA. This may be an underlying mechanism by which large luteal cells control secretion of progesterone by small luteal cells following $PGF_{2\alpha}$-induced luteolysis as progesterone production by small luteal cells is greatly enhanced by LH (Hoyer *et al.*, 1984). Clearly the mechanisms whereby large and small luteal cells communicate following $PGF_{2\alpha}$ treatment requires further investigation.

In conclusion, concentrations of mRNA encoding the receptor for LH in ovine luteal tissue increased between days 3 and 9 of the estrous cycle and decreased within 4 h of initiation of luteal regression. Small luteal cells had at least four-fold higher concentrations of mRNA encoding the receptor for LH than large luteal cells on all days examined.

Materials and methods

For both experiments, mature western range ewes were checked for estrus twice daily with the aid of vasectomized

rams. The first day of standing to be mounted by a ram (estrus) was considered day zero. All experiments were approved by the Colorado State University Animal Care and Use Committee.

Experiment 1

Corpora lutea were collected from ewes under pentobarbital anesthesia via midventral laparotomy on days 3, 6, 9, 12 and 15 of the estrous cycle; four replicates were collected on each of the specified days of the cycle. Luteal tissue was pooled from 1–7 animals for each replicate to insure that sufficient quantities of tissue were available for purification of steroidogenic cells in each pool. Corpora lutea were decapsulated, weighed, sliced at 0.5 mm with a hand-held microtome and stored at −70°C for subsequent poly(A)$^+$ RNA purification. Pools of small and large luteal cells were generated by centrifugal elutriation of enzymatically dissociated luteal tissue collected on days 6, 9, 12 and 15 ($n = 4$ per day) of the estrous cycle (Fitz *et al.*, 1982). It was not possible to purify the two luteal cell types on day 3 since large cells have not sufficiently differentiated at that stage of the estrous cycle. Purified small and large luteal cells were quantified and stored at −70°C for subsequent poly(A)$^+$ RNA purification. Blood was collected from each ewe immediately prior to surgery, allowed to clot, centrifuged and serum stored at −20°C until analysed for concentrations of progesterone by radioimmunoassay (Niswender, 1973).

Experiment 2

Ewes on day 11 or 12 of the estrous cycle were anesthetized with pentobarbital and the reproductive surgically exteriorized via a midventral incision. The ovarian artery ipsilateral to each ovary bearing corpora lutea (1–3) was identified and temporarily occluded by digital pressure. Treated ewes received an arterial infusion of 1 μmol $PGF_{2\alpha}$ (475 μg; Sigma Chemical Co., St Louis, MO) in 1 ml of vehicle [1% dimethylsulfoxide (DMSO) in saline] lasting 60 s. Typically, the corpus luteum blanched during the infusion, but quickly became pink again once circulation to the ovary was restored. This method of $PGF_{2\alpha}$ administration was chosen to allow precise timing of tissue collection relative to the initiation of the actions of $PGF_{2\alpha}$. Control animals were administered 1 ml of vehicle in the same manner. Corpora lutea were collected from $PGF_{2\alpha}$-treated animals at 1 ($n = 6$), 4 ($n = 5$), 12 ($n = 5$) and 24 h ($n = 5$) after infusion and from control animals at 0 (no infusion; $n = 3$), 12 ($n = 3$) and 24 h ($n = 4$) after infusion. Blood was collected from each ewe immediately prior to surgery and at the time of collection of corpora lutea, allowed to clot, centrifuged and serum stored at −20°C. Concentrations of progesterone in sera and luteal tissue were quantitated by radioimmunoassay (Niswender, 1973; Diekman *et al.*, 1978a).

Preparation of partial ovine LH receptor clone Following reverse transcription of 1 μg of total RNA isolated from ovine corpora lutea collected on day 10 of the estrous cycle, primers corresponding to bases 322–337 and 616–636 of the cDNA sequence for the rat LH receptor (McFarland *et al.*, 1989) were used in the polymerase chain reaction (Perkin Elmer Cetus, Norwalk, CT) to amplify a 278 base pair fragment of the ovine LH receptor cDNA. The resulting fragment was directionally cloned into the BamHI/SmaI site of Bluescript KS$^-$ plasmid (Stratagene, La Jolla, CA) and sequenced using a Sequenase Version 2.0 kit (USB, Cleveland, OH). This fragment encodes for the central one-third of the extracellular domain of the LH receptor cDNA, and encompasses all or parts of the area equivalent to exons 3 through 7 of the rat LH receptor gene (Koo *et al.*, 1994).

Poly(A)$^+$ RNA purification The procedure of Badley *et al.* (1988) was followed with modifications. Briefly, 200 mg of tissue or 7–20 million purified luteal cells were homogenized for 15–30 s with an Omni Model 2000 homogenizer (Omni International, Waterbury, CT) in 5 ml lysis buffer [prepared fresh: 200 mM NaCl, 200 mM Tris-Cl, pH 7.5, 1.5 mM $MgCl_2$ 2.0% SDS, 400 μg proteinase K/ml (Sigma Chemical Co.)] followed by incubation at 45°C with constant agitation for 60–90 min. During the incubation, Type 2 or Type 3 oligo-dT cellulose (Collaborative Research Inc., Bedford, MA) equal to 50 mg/g of tissue or 15 mg for cells was hydrated in 5 ml elution buffer (10 mM Tris-Cl, pH 7.5, 1 mM EDTA, pH 8.0). The cellulose was centrifuged for 5 s in a swinging bucket at 325 g, then washed twice with 5 ml binding buffer (500 mM NaCl, 10 mM Tris-Cl, pH 7.5, 1 mM EDTA, pH 8.0, 0.2% SDS). Following tissue incubation, 375 μl of 4 M NaCl were added to the tissue lysate and DNA was sheared by several passages through a 22-gauge needle, combined with the hydrated cellulose and rocked gently at room temperature for 20 min. The mixture was centrifuged to pellet the cellulose, the supernatant decanted, and the cellulose washed twice with 5 ml binding buffer. The cellulose was transferred to 0.45 micron filter spin columns (Millipore, Bedford, MA) using 300 μl binding buffer. The columns were centrifuged at 16 000 *g* for 5 s at room temperature, and the eluates discarded. Poly(A)$^+$ RNA was eluted from the column by addition of 300 μl elution buffer followed by centrifugation. The elution procedure was repeated, the two eluates pooled and RNA concentration determined by spectrophotometry.

RNA analysis For Northern analysis, 5–10 μg of poly(A)$^+$ RNA was fractionated in 0.8% agarose gels containing formaldehyde as described by Sambrook *et al.* (1989). To estimate sizes of mRNA species encoding the receptor for LH, 10 μg of total RNA or RNA size markers were loaded onto all gels. The RNA samples were transferred from the gels to nylon filters (Amersham, Arlington Heights, IL) by capillary blotting overnight using 10 × saline-sodium citrate (pH 7.0; 1 × = 0.15 M sodium chloride, 0.015 M sodium citrate) and fixed by u.v. crosslinking.

For slot blot analysis of mRNA encoding the receptor for LH, duplicates of each poly(A)$^+$ RNA sample (1 μg for tissue and large luteal cells, 0.5 μg for small luteal cells) were loaded onto nylon filters using a slot blot apparatus (Schleicher & Schuell, Keene, NH). Duplicate samples of ovine poly(A)$^+$ RNA isolated from day 10 corpora lutea and either ovine liver or spleen were also applied to each filter as positive and negative controls, respectively. Standards ranging from 3 pg to 600 pg of sense strand RNA transcripts of the ovine probe were also applied to each slot blot filter to allow quantitation of mRNA encoding the receptor for LH. The portion of the standard curve from 6–100 pg was linear with an R^2 value > 0.97. The densitometric measurements for all samples were within the linear range of the standard curve. All RNA samples were fixed to filters by u.v. crosslinking.

Radiolabeled ovine LH receptor probe was prepared by asymmetric PCR amplification (McCabe, 1990). Unincorporated radioactivity was removed using a Sephadex-G50 spin column. One Northern filter (surface area = 126 cm^2) or two slot blot filters (total surface area = 322 cm^2) were prehybridized in a roller-bottle hybridization chamber (Techne Inc., Princeton, NJ) for 2 h at 42°C in 10 ml of hybridization buffer (50% formamide, 5 × Denhardts', 2% SDS, 2 × SSC, and 200 μg sheared salmon sperm DNA). The LH receptor cDNA (2×10^7 c.p.m. total counts) was hybridized to filters overnight at 42°C in 10 ml of hybridization buffer. Hybridized filters were washed twice at room temperature for 20 min in 2 × SSC/0.1% SDS, then once at 65°C for 20 min in 0.5 × SSC/0.1% SDS. The washed filters were air dried and exposed to Kodak X-OMAT AR film at −70°C using two rare earth intensifying screens for 1–4 days. All filters were treated in a similar manner. Relative levels of hybridization were determined by densitometric

scanning of autoradiographs (Hoefer Scientific Instruments, San Francisco, CA).

Due to the presence of multiple sizes of mRNA transcripts encoding the receptor for LH, data from slot blots were expressed as fmol of RNA. One pg of RNA used in the standard curve contained 8.049 fmol of transcripts.

To evaluate whether similar amounts of poly(A)$^+$ RNA were loaded onto the slot blots, filters were re-hybridized to a $\gamma[^{32}P]$-ATP end-labeled oligo-dT probe (Juengel *et al.*, 1994). Since the coefficient of variation of oligo-dT binding ranged from 15% to 18%, no corrections were made for loading differences.

Statistics Data regarding concentrations of mRNA encoding the receptor for LH in luteal tissue during the course of the estrous cycle and following $PGF_{2\alpha}$ infusion were evaluated with the general linear model of SAS (Statistical Analysis Systems, Cary, NC). Differences between means were determined with Duncans' multiple range test. Concentrations of mRNA encoding the receptor for LH in small and large luteal cells were evaluated by least squares means; only predetermined comparisons between the two cell types within each day of the cycle and within each cell type across the cycle were made. In experiment 2, the three control groups collected 0, 12 and 24 h after saline infusion were not significantly different for any of the parameters measured; therefore, the data were combined into a single control group for statistical analysis.

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