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Effects of Ovariectomy and Hypothalamic-Pituitary Disconnection on Amounts of Steroidogenic Factor-1 mRNA in the Ovine Anterior Pituitary Gland

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Steroidogenic factor-1 (SF-1) is a transcription factor involved in regulation of steroidogenic enzymes. Recent evidence indicates that SF-1 is also important in the anterior pituitary gland, where it may influence gene expression in gonadotropes. We isolated a cDNA encoding ovine SF-1 and demonstrated that the SF-1 gene is expressed in the anterior pituitary gland of sheep. SF-1 transcripts and luteinizing hormone (LH) were colocalized in gonadotropes by *in situ* hybridization and immunohistochemistry, respectively. To test the hypothesis that GnRH stimulates pituitary expression of ovine SF-1 mRNA, ewes were ovariectomized to increase endogenous secretion of GnRH. Compared to ovary-intact ewes, ovariectomy resulted in three- and fourfold increases in steady-state amounts of mRNA encoding SF-1 and LHβ subunit, respectively. In ovariectomized ewes in which delivery of GnRH to the anterior pituitary gland was prevented by hypothalamic–pituitary disconnection (HPD), steady-state amounts of mRNA encoding SF-1 and LHβ-subunit were decreased. These results provide evidence that pituitary SF-1 gene expression in sheep is regulated by GnRH. Coordinate regulation of mRNAs encoding SF-1 and LHβ-subunit raises the possibility that SF-1 may be an important transcriptional regulator of LHβ-subunit gene expression in ovine gonadotropes.

Key Words: Steroidogenic factor-1; LHβ-subunit; anterior pituitary gland; gonadotrope; sheep.

Introduction

In the mammalian anterior pituitary gland, a subpopulation of cells called gonadotropes is responsible for synthesis of the gonadotrophin subunits α, LHβ, and FSHβ. These subunits are processed intracellularly to form luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which are secreted by the anterior pituitary gland and are essential for gonadal function. Synthesis and secretion of the gonadotrophins require stimulation of the anterior pituitary gland by hypothalamic GnRH, which interacts with specific cell-surface receptors on gonadotropes. Thus, reproductive success depends upon coordinated regulation of several genes expressed at the level of the gonadotrope. However, molecular mechanisms underlying regulation of genes encoding gonadotrophin subunits and the GnRH receptor are not completely defined.

Steroidogenic factor-1 (SF-1), an orphan member of the nuclear receptor superfamily, is a transcription factor essential for regulation of cytochrome P-450 steroid hydroxylase gene expression in the gonads and adrenal cortex *(1)*. Recent evidence indicates that effects of SF-1 on reproductive function are not limited to regulation of gonadal steroidogenesis, but are exerted at multiple levels of the hypothalamic–pituitary–gonadal axis. The importance of SF-1 in nonsteroidogenic reproductive tissues was first demonstrated by targeted disruption of the FTZ-F1 gene encoding SF-1 in mice. This disruption not only prevented gonadal and adrenal development *(2)*, but also resulted in a lack of gonadotrope-specific markers in the anterior pituitary gland *(3)* and gross structural impairment of the ventromedial hypothalamic nucleus *(4)*. Thus, SF-1 appears to be critical for normal development of several components of the reproductive axis, including the gonadotrope.

In addition to its potential role during development of gonadotropes, SF-1 may also be an important transcriptional regulator of gonadotrope-specific genes in the mature anterior pituitary gland. Interaction of SF-1 with a consensus binding site and transcriptional activation has been demonstrated in human α-subunit *(5)*, rat LHβ-subunit *(6)*, bovine LHβ-subunit *(7)*, and murine GnRH receptor *(8)* promoters. Conservation of this response element in at least three genes expressed by gonadotropes across several different

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species leads to the intriguing possibility that SF-1 may be a common transcriptional regulator of genes that define the mammalian gonadotrope. Consistent with this possibility is evidence for SF-1 gene expression in rat *(3)* and human *(9)* gonadotropes.

In vivo, levels of expression of α-subunit, LHβ-subunit, and GnRH receptor genes are stimulated by GnRH *(10,11)*. If SF-1 mediates transcriptional activation of these genes, then expression of the SF- 1 gene may also be regulated in a positive fashion by GnRH; indeed, evidence for such regulation has recently been reported in the rat *(12)*. Additional evidence for regulation of SF-1 gene expression by GnRH in other species would strengthen the possibility that SF-1 is involved in the molecular mechanisms underlying stimulatory effects of GnRH on gonadotrophin subunit gene expression.

The present studies were designed to explore expression of the gene encoding SF-1 in the ovine anterior pituitary gland. The sheep is an excellent experimental model to study regulation of pituitary gene expression, since the large size of the ovine anterior pituitary gland allows analysis of multiple gene products within the same animal. Furthermore, surgical procedures involving the hypothalamic–pituitary axis are routine in this species. Our first objective was to isolate a partial cDNA encoding ovine SF-1 to determine whether SF-1 mRNA is expressed in the anterior pituitary gland of sheep. Our second objective was to test the hypothesis that changes in endogenous secretion of GnRH are associated with coordinate alterations in pituitary expression of mRNA encoding SF-1 and LHβ subunit.

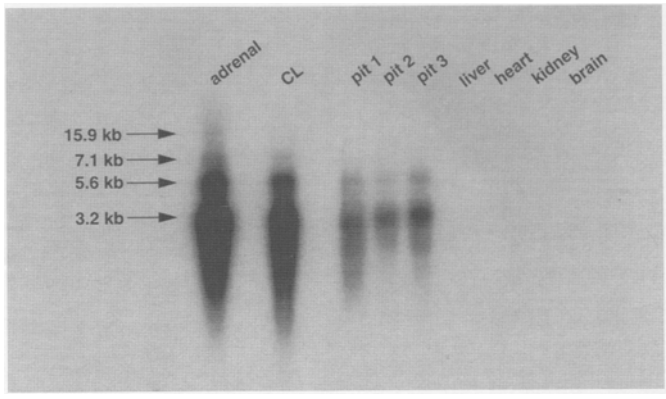

Fig. 1. Northern blot analysis of ovine RNA. Ten micrograms of poly(A)+ RNA from tissues indicated across the top were hybridized to radiolabeled ovine SF-1 cDNA. Estimated sizes of SF-1 mRNA transcripts are shown on the left.

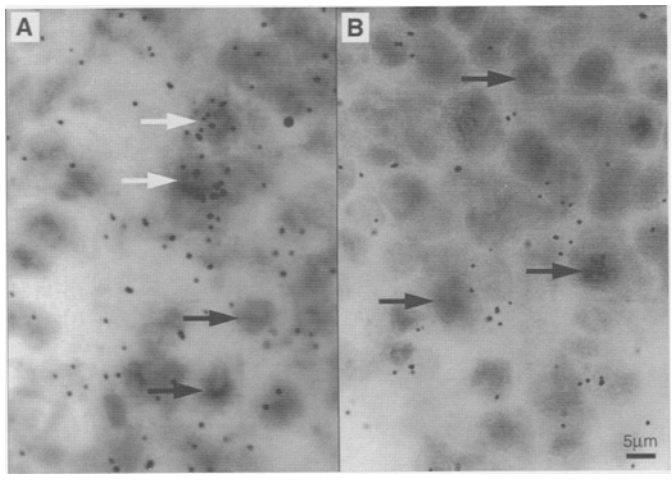

Fig. 2. *In situ* hybridization and immunohistochemistry in ovine anterior pituitary tissue. Ten-micrometer cryosections were hybridized with ovine SF-1 cRNA probes and analyzed immunohistochemically with antiserum specific for LH. **(A)** Hybridization with antisense SF-1 cRNA probe. White arrows denote 2 cells that are immunopositive for LH (indicated by dark color) and express SF-1 mRNA (indicated by silver grains). Black arrows denote cells that contain immunoreactivity for LH, but no evidence of SF-1 mRNA. **(B)** Hybridization with sense SF-1 cRNA probe. Black arrows denote cells that are immunopositive for LH. Bar = 5 μm.

Results

Nucleotide sequence of the 428 bp cDNA was approx 97 and 88% homologous to bovine Ad4BP and murine SF-1, respectively *(1,13)*. The cDNA corresponded to base pairs 877–1304 of bovine Ad4BP (entire length of the open reading frame, 1383 bp; *13*). In Northern blot analysis, the ovine SF-1 cDNA hybridized to four mRNA transcripts in adrenal cortex at approx 15.9, 7.1, 5.6, and 3.2 kb (Fig. 1). Of these transcripts, all but the largest were also observed in corpus luteum. In RNA from anterior pituitary glands from 3 different ewes, 2 SF-1 transcripts were evident at 5.6 and 3.2 kb. There was no apparent hybridization of SF-1 cDNA to RNA from ovine liver, heart, kidney, or brain.

In situ hybridization combined with immunohistochemistry revealed the presence of SF-1 mRNA transcripts in cells containing LH in ovine anterior pituitary tissue (Fig. 2). However, SF-1 mRNA was not detectable in all cells that were immunopositive for LH.

Relative amounts of pituitary mRNA encoding SF-1 and LHβ subunit are illustrated in Fig. 3. Compared to intact ewes, greater amounts of SF-1 mRNA ($p < 0.001$) were detected in long-term OVX ewes. In OVX-HPD ewes 48 and 72 h after HPD, amounts of SF-1 mRNA were decreased ($p < 0.001$) relative to OVX ewes and were also lower ($p < 0.02$) than amounts in intact ewes. Amounts of LHβ subunit mRNA were higher ($p < 0.001$) in OVX ewes than in intact ewes. In OVX- HPD ewes 48 h after HPD, amounts of LHβ subunit mRNA were decreased ($p < 0.001$) relative to OVX ewes, but were higher ($p < 0.01$) than amounts in intact ewes. By 72 h after HPD, amounts of LHβ subunit mRNA in OVX-HPD ewes were similar ($p > 0.05$) to those in intact ewes and tended to be lower

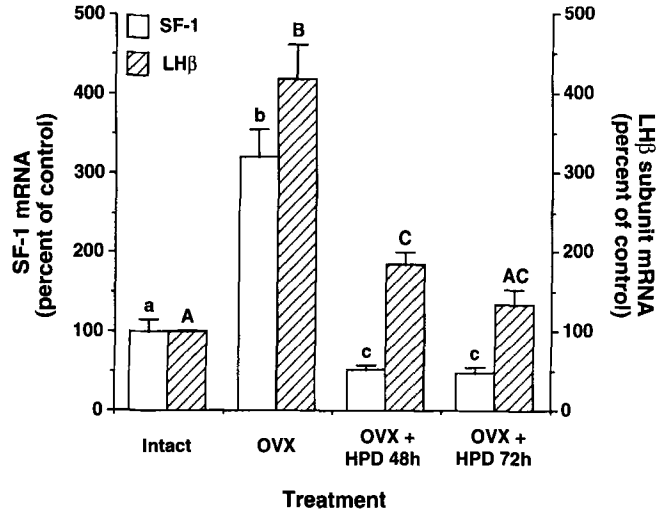

Fig. 3. Steady-state amounts (mean ± SEM) of mRNA encoding SF-1 or LHβ subunit in pituitary glands of ovary-intact ewes (intact), long-term OVX ewes, and OVX ewes that had been HPD for 48 or 72 h. Data are expressed as percent of intact control values. Significant differences ($p < 0.02$) among means are indicated by lower-case letters for SF-1 mRNA and upper-case letters for LHβ subunit mRNA.

($p < 0.06$) than amounts in OVX-HPD ewes 48 h after HPD. Across all groups, amounts of SF-1 mRNA and LHβ subunit mRNA were correlated ($r = 0.92$, $p < 0.001$).

Discussion

In addition to the critical role of SF-1 in development and function of steroidogenic tissues, evidence is accumulating to support a role of SF-1 in regulation of gene expression in the anterior pituitary gland, specifically in the subpopulation of cells responsible for synthesis and secretion of gonadotropic hormones. Herein, we describe isolation of a partial cDNA encoding ovine SF-1. The identity of this cDNA was confirmed by a high degree of nucleotide sequence homology compared to bovine Ad4BP (the bovine form of SF-1; *13,14*) and murine SF-1 *(1)*. In the mouse, SF-1 is the product of the FTZ-F1 gene. However, alternative splicing of FTZ-F1 transcripts generates another protein, embryonal long-terminal repeat binding protein (ELP; *1*). Although homology of the partial cDNA described in this article initiates in a coding sequence common to SF-1 and ELP, it terminates in a coding sequence unique to SF-1 and contains no ELP-unique sequence *(1)*. Consistent with its role in mediating expression of steroidogenic enzymes, ovine SF-1 cDNA hybridized to several mRNA transcripts in ovine adrenal cortex and corpus luteum. Multiple SF-1 mRNA transcripts have also been reported for other species *(5,13)*. In ovine pituitary RNA, two sizes of SF-1 transcripts were detected at lower abundance relative to adrenal and luteal tissue. If expression of SF-1 is primarily confined to gonadotropes, then lower abundance of SF-1 mRNA in pituitary tissue likely reflects the heterogenous cellular composition of the pituitary gland, of which gonadotropes comprise approx 10% *(15)*. Expression of the SF-1 gene has been found in pituitaries of rodents *(3,5)* and humans *(9)*, and the present study extends these observations to include a domestic ruminant, the sheep. The most abundant RNA transcript in both steroidogenic and pituitary tissues of ewes was 3.2 kb in size, and this corresponds with results in murine pituitary gland in which the most abundant transcript was 2.9 kb.

To determine whether the SF-1 gene is expressed in ovine gonadotropes, we combined *in situ* hybridization for SF-1 mRNA and immunohistochemistry for LH. In agreement with previous evidence in the rat *(3)*, our results indicate that SF-1 transcripts and LH are colocalized in ovine anterior pituitary tissue. However, SF-1 transcripts were not detectable in all ovine cells that were immunopositive for LH. It is possible that the gene for SF-1 is not expressed in every ovine gonadotrope, or that it is expressed transiently. Alternatively, the apparent lack of SF-1 transcripts in some LH-containing cells may simply reflect the limit of detection of the *in situ* hybridization technique. Indeed, the fact that several micrograms of poly (A)+ RNA are needed to obtain a signal in Northern blot analysis (*see* Fig. 1) indicates that the gene encoding ovine SF-1 is expressed at low levels in the pituitary gland relative to the gonadotrophin subunit genes. Because we did not attempt to co-localize SF-1 mRNA and other pituitary hormones, we cannot preclude the possibility that SF-1 mRNA may also be present in pituitary cell subpopulations other than gonadotropes. In the human pituitary gland, SF-1 protein was detected only in cells containing gonadotrophin β-subunits (*9*), indicating that at least in this species, expression of the SF-1 gene is limited to gonadotropes. Since none of the studies reported to date have involved a quantitive histological approach to the relationship between SF-1 gene expression and gonadotrope-specific markers, the question of whether all gonadotropes have the capacity to express the SF-1 gene is difficult to answer with certainty. However, colocalization of SF-1 mRNA and LH in the ovine pituitary gland indicates that like the rat and human, expression of the SF-1 gene is a characteristic of ovine gonadotropes. This finding raises the possibility that SF-1 may act in a cell-specific manner to regulate gene expression in the ovine gonadotrope.

Recent studies have indicated that SF-1 may be essential for development of gonadotropes *(3)* and the ventromedial hypothalamus *(4)*, and SF-1 may regulate expression of genes encoding α subunit, LHβ-subunit, and GnRH receptor (*5,7,8*, respectively). However, there have been few reports describing factors that regulate pituitary expression of the SF-1 gene itself. The present study provides compelling evidence that in the sheep, expression of the gene encoding SF-1 in the anterior pituitary gland is controlled at least in part by hypothalamic GnRH. Long-term ova-

riectomy was accompanied by a greater than threefold increase in steady-state amounts of SF-1 mRNA. It seems likely that this increase in amounts of SF-1 mRNA was caused by heightened secretion of GnRH as a result of removal of steroid negative feedback *(16)*, since depriving the pituitary gland of GnRH by HPD decreased amounts of SF-1 mRNA. Amounts of SF-1 mRNA following HPD were even lower than those in intact ewes, and this difference can be attributed to the presence of pulsatile GnRH in intact ewes (although at a lower frequency than in ovariectomized ewes). The close relationship between endogenous secretion of GnRH and steady-state amounts of SF-1 mRNA leads us to postulate that expression of the gene encoding SF-1 in the ovine anterior pituitary gland is stimulated by GnRH. This claim is also supported by a recent study in which increased levels of SF-1 mRNA were observed in rats following ovariectomy or treatment of GnRH-deficient females with pulsatile GnRH *(12)*. Future studies in which pulsatile stimulation of the pituitary gland by GnRH is restored in HPD ewes will provide definitive evidence for GnRH regulation of pituitary SF-1 gene expression.

It is interesting that amounts of SF-1 mRNA were still detectable following HPD. Levels of expression of the LHβ-subunit and α-subunit genes, which are also GnRH-dependent, are decreased 3 d after HPD and continue to fall toward nondetectable levels by 7 d *(17)*. Therefore, it is possible that SF- 1 mRNA might also disappear completely with additional time after withdrawal of GnRH. Alternatively, residual amounts of SF-1 mRNA could be indicative of a component of SF-1 gene expression that is independent of stimulation by GnRH.

As previously observed, removal of ovarian negative feedback increased *(18)* and HPD decreased *(19)* steady-state amounts of LHβ-subunit mRNA. Most interesting is the finding that changes in LHβ-subunit gene expression occurred in concert with alterations in amounts of SF-1 mRNA, and it is interesting to speculate that the more rapid decrease in amounts of SF-1 mRNA may be a prerequisite for the decrease in levels of LHβ-subunit mRNA following removal of GnRH by HPD. Although the present experiment was designed to study steady-state levels of mRNA encoding SF-1 and LHβ-subunit, coordinate changes in amounts of these mRNAs raise the possibility that SF-1 may be a transcription factor involved in expression of the LHβ-subunit gene in sheep. More direct evidence for SF-1 regulation of LHβ subunit gene expression in a species closely related to the sheep is SF-1-induced, binding site-dependent trans-activation of the bovine LHβ-subunit promoter in αT3-1 cells *(7)*. Moreover, mutation of the SF-1 binding site in the LHβ promoter greatly attenuated its activity in transgenic mice *(7)*. Thus, transcriptional regulation of the ovine LHβ-subunit gene by SF-1 is a distinct possibility that deserves further attention. In addition to decreased transcriptional activity, mutation of the SF-1 binding site also eliminated responsiveness of the bovine LHβ promoter to physiological concentrations of GnRH in transgenic mice *(7)*. Although this observation provides evidence that the presence of SF-1 may be necessary to mediate effects of GnRH on LHβ subunit gene expression, it should be noted that treatment of SF-1 "knockout" mice with pharmacological doses of GnRH restored gonadotrophin expression and thus compensated for the lack of SF-1 *(4)*. Therefore, although SF-1 might enhance transcription of gonadotrophin subunit genes, it may not be the sole factor involved in mediating effects of GnRH on gene expression in gonadotropes.

In summary, we have isolated a partial cDNA encoding ovine SF-1 and have demonstrated SF-1 gene expression in the anterior pituitary gland of sheep. SF-1 mRNA was present in gonadotropes of the ovine anterior pituitary gland, and manipulation of endogenous concentrations of GnRH by ovariectomy and HPD resulted in coordinate changes in steady-state levels of mRNA encoding SF-1 and LHβ subunit. Based on these results, we suggest that ovine SF-1 gene expression is stimulated by GnRH, and that SF-1 may be important in mediating GnRH-stimulated increases in gonadotrophin subunit gene expression in the ewe.

Materials and Methods

cDNA Cloning and Northern Blot Analysis

To isolate a partial cDNA encoding ovine SF-1, total cellular RNA was isolated from corpora lutea collected from ewes on d 10 of the estrous cycle *(20)*. Reverse transcription of this RNA and amplification of SF-1 cDNA by polymerase chain reaction (PCR) were performed using procedures identical to those reported for isolation of bovine adrenal four binding protein (Ad4BP, the bovine form of SF-1) by Honda et al. *(13)*. Briefly, 1 μg luteal RNA was reverse transcribed using random hexanucleotides. Products of this reaction were subjected to PCR using 19-mer oligonucleotide primers (sense, 5'-GACCAGATGACACTGCTGC-3'; antisense, 5'-TCCTTGGCCTGCATGCTCA-3'). The amplified cDNA fragment was expected to be 428 bp in length and correspond to base pairs 877–1304 of bovine Ad4BP. This partial cDNA was subcloned into pGEM-T vector (Promega, Madison, WI), and its nucleotide sequence was determined by automated sequence analysis (Macromolecular Resources, Colorado State University, Fort Collins, CO).

To examine tissue distribution and sizes of SF-1 mRNA transcripts, the 428-bp partial cDNA was used as a probe in Northern blot analysis. Polyadenylated (poly[A]+) RNA was prepared *(21)* from ovine adrenal cortex, corpus luteum, anterior pituitary gland, liver, heart, kidney, and brain. Ten micrograms of poly(A)+ RNA were separated by electrophoresis in a 1% denaturing agarose gel and transferred to nylon membrane (Hybond-N; Amersham,

Arlington Heights, IL) by capillary action. RNA size markers were included on the gel (Promega, Madison, WI), and sizes of mRNA transcripts were estimated from these markers. RNA was crosslinked to the nylon by exposure to uv radiation (UVP Inc., Upland, CA). The membrane was prehybridized overnight and hybridized for 24 h with radiolabeled SF-1 cDNA at 42°C in a roller-bottle hybridization oven (Techne Inc., Princeton, NJ). The hybridization solution consisted of 50% formamide, 0.5% SDS, 0.1 mg/mL denatured salmon sperm DNA, 0.02 *M* piperazine-*N*-*N*' bis[2- ethane-sulfonic acid] (PIPES), 0.8 *M* NaCl, and 0.002 *M* EDTA. The SF-1 cDNA was radiolabeled with [^{32}P] deoxy-CTP (3000 Ci/mmol; Amersham, Arlington Heights, IL) by the random hexamer priming method (Boehringer Mannheim, Indianapolis, IN). The final wash following hybridization was in 0.5X SSC, 0.1% SDS at 65°C. The membrane was exposed to Hyperfilm-MP (Amersham) for 24 h.

In Situ *Hybridization and Immunohistochemistry*

In situ hybridization was used to localize SF-1 mRNA in pituitary tissue. Plasmid containing the 428-bp cDNA insert partially encoding ovine SF-1 was linearized with *Spe*I or *Nco*I (Promega, Madison, WI), and antisense and sense riboprobes were synthesized in the presence of [^{35}S]UTP with T7 or SP6 RNA polymerase (Promega), respectively *(22)*. Ten micrometer cryosections of anterior pituitary tissue obtained from an ovariectomized ewe were thaw-mounted on Superfrost slides (Fisher Scientific, Denver, CO). As a positive control for the presence of SF-1 mRNA transcripts, cryosections of ovine steroidogenic tissue (corpus luteum) were also subjected to *in situ* hybridization. Slides were processed successively through 4% paraformaldehyde (pH 7.2), 2X SSC, distilled H_2O, 0.1 *M* triethanolamine (pH 8.0), 0.1 *M* triethanolamine containing 0.25% acetic anhydride, and 2X SSC. Tissue sections were then progressively dehydrated in 50, 75, 95 and 100% ethanol and allowed to air-dry. Seventy microliters of hybridization buffer (50% formamide, 0.3 *M* NaCl, 10 m*M* Tris, 1 m*M* EDTA, 1X Denhardt's solution, 10% dextran sulfate) containing 2×10^7 cpm/mL riboprobe were placed on each slide and overlayed with a coverslip. Hybridization was allowed to proceed overnight at 42°C in a humid chamber. Slides were washed in 2X SSC to remove coverslips before treatment with RNase A (20 µg/mL 2X SSC) for 30 min at 37°C. Following a stringent wash in 0.1X SSC containing 12.5 m*M* β-mercaptoethanol and 1m*M* EDTA, slides were transferred to PBS for immunostaining.

To determine whether SF-1 transcripts are localized to gonadotropes, LH-containing cells were identified immunohistochemically after *in situ* hybridization. Slides were transferred from PBS to 0.25% H_2O_2 in PBS for 5 min to inactivate endogenous peroxidases. Nonspecific antibody binding sites were blocked by incubation with 10% normal swine serum diluted in PBS containing 0.05% Triton X-100. Primary antiserum, rabbit anti-ovine LH, was diluted 1:100 in antibody dilution buffer (PBS containing 0.3% Triton X-100), and incubated with tissue sections for 1 h at 37°C. Additional slides were incubated with nonimmune rabbit serum (diluted 1:100 in antibody dilution buffer). Specificity of this antiserum for LH has been demonstrated previously *(15)*. Slides were washed twice in PBS before incubation with swine antirabbit γ-globulin conjugated to horseradish peroxidase (diluted 1:100 in antibody dilution buffer; DAKO Corp., Carpinteria, CA) for 30 min at room temperature. Slides were washed again in PBS before addition of 3,3' diaminobenzidine (DAB: Sigma Chemical Co., St. Louis, MO). Once color was observed, slides were returned to PBS, and then dehydrated through 50, 75, 95, and 100% ethanol containing 0.3 *M* ammonium acetate. Slides were air-dried and dipped in NTB-2 photographic emulsion (Eastman Kodak, Rochester, NY). Exposure time was 4 wk at 4°C. Slides were developed with D-19 developer (Eastman Kodak), stained lightly with hematoxylin, dehydrated again, and coverslipped using Permount mounting medium (Fisher, Scientific, Denver, CO).

Animals and Experimental Design

Removal of steroid negative feedback effects by ovariectomy leads to increased endogenous secretion of GnRH in sheep *(16)*. Therefore, to test whether increased secretion of GnRH leads to changes in pituitary expression of ovine SF-1 mRNA, anterior pituitary glands were collected from four sexually mature, ovary-intact ewes on d 10 of the estrous cycle and from four ovariectomized (OVX) ewes. To study effects of decreased stimulation of the anterior pituitary gland by GnRH, 7 OVX ewes were subjected to hypothalamic–pituitary disconnection (HPD; *23*). This procedure surgically disconnects the hypothalamus from the pituitary gland, thus preventing transport of GnRH to the hypophysis, but leaves the hypophyseal blood supply intact. Ovariectomized-HPD ewes received no further treatment and their pituitary glands were collected 48 h ($n = 3$) or 72 h ($n = 4$) following the HPD procedure. These ewes were also included in previous experiments *(19,24)*. Ewes were ovariectomized at least 8 wk before experiments were conducted. Pituitary glands were collected following anesthesia with sodium pentobarbital and exsanguination. Pituitary tissues were frozen immediately on dry ice and stored at –80°C. All procedures involving animals were approved by the Colorado State University Animal Care and Use Committee and complied with NIH guidelines.

Quantitation of mRNA

Poly (A)+ RNA was prepared from pituitary tissues, and its integrity was confirmed by Northern blot analysis as described above. To quantitate steady-state amounts of mRNAs, 5 µg RNA were applied to nylon membranes in duplicate using a slot-blot apparatus. Hybridization to

radiolabeled SF-1 cDNA was carried out as described above, and membranes were washed at a final stringency of 0.5X SSC, 0.1% SDS at 65°C. Membranes were exposed to film for 3-11 d. Autoradiographs were analyzed using the NIH 1.52 Image Analysis Program before being stripped of SF-1 cDNA by washing in boiling 0.1% SDS. Membranes were rehybridized to bovine LHβ-subunit cDNA *(25)*, which was radiolabeled by the same method used for SF-1 cDNA. Following autoradiography for 4–6 h and analysis of autoradiographs, membranes were stripped a second time and probed with radiolabeled dT (18-mer) as described previously *(26)*. This procedure allows for normalization of unequal loading among RNA samples on the nylon membrane. Relative steady-state concentrations of mRNA encoding SF-1 or LHβ-subunit are expressed as percentages relative to respective amounts in intact ewes.

Statistical Analysis

Differences in steady-state amounts of mRNA encoding SF-1 or LHβ-subunit among intact, OVX and OVX-HPD ewes were determined by one-way analysis of variance (ANOVA) using the general linear model procedure of SAS *(27)*. Data were log-transformed to correct for heterogeneity of variance. Means were separated using least significant differences *(27)*. Correlation between amounts of mRNA encoding SF-1 and LHβ-subunit across groups is expressed as Pearson Correlation Coefficient (*r*). All quantitative data are presented as mean ± SEM.

Acknowledgments

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