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Differential Regulation of Gonadotropin Synthesis and Release in Ovariectomized Ewes after Treatment with a Luteinizing Hormone-Releasing Hormone Antagonist[1]

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ABSTRACT

Our working hypothesis was that synthesis and release of LH, but not FSH, were solely dependent on LHRH. Twenty ovariectomized (OVX) ewes were randomly assigned to one of five treatments (n = 4 per group). Ewes were administered a low (10 μg/kg) or high (100 μg/kg) dose of LHRH antagonist (LHRH-Ant) at 24-h intervals for 3 or 6 days. Control ewes received vehicle (5% mannitol) at 24-h intervals for 6 days. Blood samples were collected every 15 min for 4 h before LHRH-Ant or vehicle and every 2 h during the period of treatment to determine concentrations of LH and FSH. Twenty-four hours after the last treatment with LHRH-Ant or vehicle, anterior pituitaries were collected and divided in half along the midsagittal plane; the number of receptors for LHRH, pituitary content of LH and FSH, and relative amounts of mRNA for α, LHβ, and FSHβ subunits were determined. Concentrations of LH in serum decreased ($p < 0.05$) from 25.4 ± 4.3 ng/ml before LHRH-Ant to less than 0.5 ng/ml within 4 h after the first treatment of LHRH-Ant and remained low (< 0.5 ng/ml) throughout the study. Serum concentrations of FSH declined gradually during the 3- or 6-day period of treatment with LHRH-Ant, from 37.3 ± 2.4 and 26.5 ± 4.8 ng/ml to 19.9 ± 1.8 and 13.7 ± 2.1 ng/ml, respectively. The magnitude of decline in serum concentrations of LH and FSH did not differ among ewes treated with low or high doses of LHRH-Ant. Pituitary content of LH was not different ($p > 0.10$) from that in controls, whereas pituitary content of FSH was greater ($p < 0.01$) in control ewes compared to ewes treated with LHRH-Ant. Receptors for LHRH were nondetectable (< 0.018×10^{-16} mol receptor/μg protein) in pituitaries after 3 or 6 days of treatment with LHRH-Ant (low or high dose). Relative amounts of mRNA for α, LHβ, and FSHβ subunits were lower ($p < 0.01$) after 6 days of treatment with LHRH-Ant (low or high dose) than after 3 days of treatment with LHRH-Ant (low or high dose). The LHRH was, therefore, required to maintain steady state amounts of mRNA for FSH and LH and to maintain pituitary stores of FSH but not LH. Our data support the hypothesis that differential regulation of LH and FSH release occurs in ewes. While synthesis and release of LH are dependent on LHRH, synthesis but not release of FSH appears to be dependent on LHRH.

INTRODUCTION

After characterization of LHRH, it was widely accepted that LHRH not only stimulates release of LH, but also FSH, from gonadotrophs of the anterior pituitary [1]. There are some reports suggesting that factors other than LHRH may regulate release of FSH [2–5]. The role of LHRH in modulation of the release of FSH was questioned because decreased stimulation of the pituitary resulted in a greater decrease in release of LH than in release of FSH.

One method of studying the physiology of the isolated pituitary gland is to actively immunize ovariectomized ewes against LHRH to abolish the specific hypothalamic input. In ovariectomized ewes immunized against LHRH, it has been reported that the release of both LH and FSH is markedly reduced [6].

Another method to isolate the pituitary from the influence of the hypothalamus is to perform the surgical procedure of hypothalamic-pituitary disconnection. In sheep, hypothalamic-pituitary disconnection leaves the primary blood supply (superior hypophyseal arteries) to the pars distalis intact, while the pituitary gland remains viable and functions independently of hypothalamic input [7]. After hypothalamic-pituitary disconnection in ovariectomized ewes, pituitary and serum concentrations of LH and FSH as well as amounts of mRNA for the subunits of gonadotropins were reported to decline within 3 days [4].

A third method for studying the physiology of the pituitary is through the use of antagonists against LHRH. Synthetic LHRH antagonists inhibit gonadotropin release by occupation of LHRH receptors on gonadotrophs [5, 8–10]. Use of LHRH antagonists is advantageous to hypothalamic-pituitary disconnection because only LHRH inputs are blocked, leaving the rest of the hypothalamic-pituitary axis intact. The objective of the present study was to use an LHRH antagonist to determine the role of LHRH in the regulation of synthesis and release of LH and FSH in ovariectomized ewes.

MATERIALS AND METHODS

Twenty crossbred ovariectomized ewes were randomly assigned to one of five groups for treatment with either a low (10 μg/kg) or a high (100 μg/kg) dose of an LHRH

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TABLE 1. Concentrations of anterior pituitary receptors for LHRH.

Treatment	Receptor concentrations (10^{-16} mol/μg protein)
Control	1.36 ± 0.44[d]
Ant-3Low[a]	ND[c]
Ant-3High[a]	ND
Ant-6Low[b]	ND
Ant-6High[b]	ND

[a]Low (10 μg/kg) or High (100 μg/kg) concentration of LHRH-antagonist for 3 days.
[b]Low (10 μg/kg) or High (100 μg/kg) concentration of LHRH-antagonist for 6 days.
[c]ND = Nondetectable, <0.018 × 10^{-16} mol receptor/μg protein.
[d]Mean ± SEM.

antagonist (LHRH-Ant). The antagonist used in this study was [*N*-Ac-D-Nal(2)'-4Cl-D-Phe^2, D-Pal(3)^3-D-Cit^6, D-Ala^{10}]-LHRH (SB-75). The LHRH-Ant was dissolved in 5% mannitol, and 1 ml was administered s.c. Ewes received the low or high dose of LHRH-Ant every 24 h for 3 (n = 4; Ant-3Low and Ant-3High, respectively) or 6 days (n = 4; Ant-6Low and Ant-6High, respectively). Ewes in the control group received sham injections (1 ml 5% mannitol) every 24 h for 6 days. In sheep, the low concentration of LHRH-Ant (10 μg/kg) had been shown previously to suppress release of LH for 24 h when administered s.c. [11]. The high concentration of LHRH-Ant (100 μg/kg) was used to ensure that all receptors to LHRH would be occupied.

Blood samples were collected every 2 h from 4 h before the initiation of treatment until 24 h after the last injection. Blood was allowed to clot at room temperature and stored at 4°C for 12 h; serum was then separated by centrifugation at 1500 × *g* for 15 min. Serum was stored at −20°C until assayed for LH and FSH.

Quantitation of LH [12] and FSH [13, 14] in serum and pituitaries was by RIA. Intraassay coefficients of variation were 4.3 and 4.2%, and interassay coefficients of variation were 11.1 and 9.7% for LH and FSH, respectively.

Twenty-four hours after the last treatment with LHRH-Ant, animals were administered 4 ml of phenobarbital to achieve general anesthesia. Anterior pituitary glands were collected immediately after exsanguination, divided in half along the midsagittal plane, and stored at −70°C until subsequent analysis. Half of each anterior pituitary gland was used to measure receptors for LHRH [15] and pituitary content of LH and FSH as described above. The remaining half of each anterior pituitary gland was used to measure steady state amounts of mRNA for α and LHβ subunits [16], and a similar procedure was followed for FSHβ subunit. The remaining half of each pituitary gland was used to isolate total cellular RNA as described previously [4, 16]. Five micrograms of total cellular RNA from individual hemipituitaries was separated by electrophoresis through 1.5% agarose gels containing formaldehyde and transferred to nylon membranes (Zeta-Probe GT; Bio-Rad Labs., Hercules, CA).

Radioactive cDNA probes for bovine α subunit [17], bovine LHβ subunit [18], bovine FSHβ subunit [19], or rat cyclophilin [20] were prepared by random-primed labeling [21]. Specific activity of all radioactive cDNA probes was approximately 2 × 10^9 dpm/μg. Radioactive cDNA probes (10^6 cpm/ml; 10 ml/nylon membrane) were hybridized to total cellular RNA on nylon membranes for at least 24 h at 42°C and washed; specific radiolabeled bands were visualized by autoradiography for 24–96 h at −70°C [4]. The relative density of each band was quantitated by scanning densitometry with a Visage 110 image analyzer (Eastman Kodak, Rochester, NY). Relative amounts of mRNA for gonadotropin subunits were normalized to relative amounts of cyclophilin mRNA to correct for unequal loading of RNA [16]. Values are expressed as percentage of those measured in ovariectomized, control ewes (ovariectomized = 100%).

Concentrations of LH and FSH in circulation before and after treatment with LHRH-Ant, pituitary contents of LH and FSH, and relative amounts of mRNA for gonadotropin subunits were examined by analysis of variance using the general linear models procedure of the SAS program [22]. Differences between treatment means were determined by Fisher's least significant difference test [22, 23].

RESULTS

Receptors for LHRH in anterior pituitary glands were nondetectable (< 0.018 × 10^{-16} mol receptor/μg protein) after 3 or 6 days of treatment with LHRH-Ant (either low or high dose). Control ewes had 1.31 ± 0.44 × 10^{-16} mol receptor/μg protein (Table 1).

With both the low and high doses of LHRH-Ant, concentrations of LH in serum decreased ($p < 0.01$) from an average of 25.4 ± 4.3 ng/ml before administration of LHRH-Ant to less than 2.0 ng/ml within 8 h after the first treatment of LHRH-Ant (Fig. 1). Concentrations of LH remained low (< 0.5 ng/ml) throughout the period (3 or 6 days) of treatment (Table 2).

Concentrations of FSH in serum declined gradually, to 44% of the original concentration, in ewes from the Ant-3Low and Ant-3High groups (Fig. 1). Similarly, there was a 52% decrease in concentrations of FSH in ewes treated with low or high concentrations of LHRH-Ant for 6 days. The magnitude of decline in serum concentrations of FSH did not differ among ewes treated with the low vs. the high dose of LHRH-Ant. Concentrations of FSH in serum after 3 or 6 days of treatment with LHRH-Ant were lower ($p < 0.01$) than in controls. Pituitary content (mg/pituitary) of LH was not different ($p > 0.05$) in ewes treated with LHRH-Ant compared to control ewes. Amounts of LH were, however, greater ($p < 0.05$) in ewes from the Ant-6Low (1.54 ± 0.44) group than in those from the Ant-6High group (0.71 ± 0.10). Pituitary content (mg/pituitary) of FSH was greater ($p < 0.01$) in controls (0.75 ± 0.25) than in ewes from the Ant-3Low (0.13 ± 0.02), Ant-3High (0.19 ± 0.06), Ant-6Low (0.14 ± 0.04), and Ant-6High (0.14 ± 0.04) groups (Fig. 2).

Steady state amounts of mRNA for α and LHβ subunits were different ($p < 0.01$) among all treatment groups and

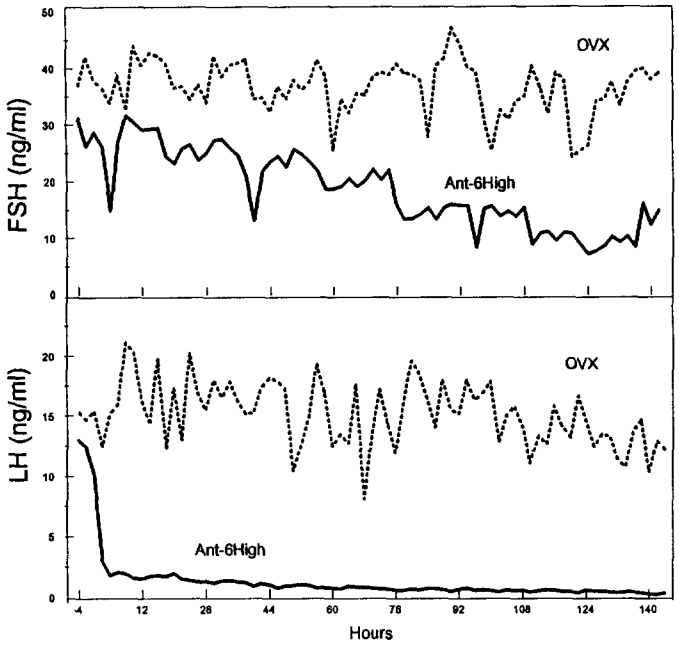

FIG. 1. Concentrations of LH and FSH during administration of LHRH antagonist or vehicle. The top panel depicts mean concentrations of serum FSH in control ewes (dashed line, OVX) and ewes in the Ant-6High group (solid line). Mean concentrations of LH are depicted in the bottom panel for control ewes (dashed line, OVX) and ewes in the Ant-6High group (solid line). Ewes in the Ant-6 group were treated with 100 μg/kg LHRH-Ant at 24-h intervals for 6 days.

were dose- and time-dependent (α: 36.33, 29.16, 18.25, and 12.13% of control for Ant-3Low, Ant-3High, Ant-6Low, and Ant-6High groups, respectively; LHβ:70.17, 68.37, 29.76, and 25.50% of control for Ant-3Low, Ant-3High, Ant-6Low, and Ant-6High, respectively). Amounts of mRNA for FSHβ subunit were lower ($p < 0.01$) in pituitaries from all treatment groups compared to controls. Amounts of mRNA for FSHβ also decreased in a dose- and time-dependent manner (79.59, 60.64, 67.10, and 36.04% of control for Ant-3Low, Ant-3High, Ant-6Low, and Ant-6High treatments, respectively, Fig. 3).

DISCUSSION

Effects of LHRH on synthesis and release of LH and FSH were examined by administering a low (10 μg/kg) or a high (100 μg/kg) dose of LHRH-Ant for 3 or 6 days. The low dose of LHRH-Ant had been previously used to suppress release of LH in wethers [11].

Receptors for LHRH were below the level of assay detection in ewes treated with either dose of LHRH-Ant in the present study. The LHRH-Ant, SB-75, binds to a population of high-affinity receptors for LHRH in membranes of pituitaries [24, 25]. Binding of SB-75 to receptors for LHRH in pituitaries might influence the rate of turnover of the plasma membrane [26] and the rate of internalization of LHRH receptors [27]. Prolonged treatment with SB-75 (60 days) was reported to result in down-regulation of LHRH receptors in

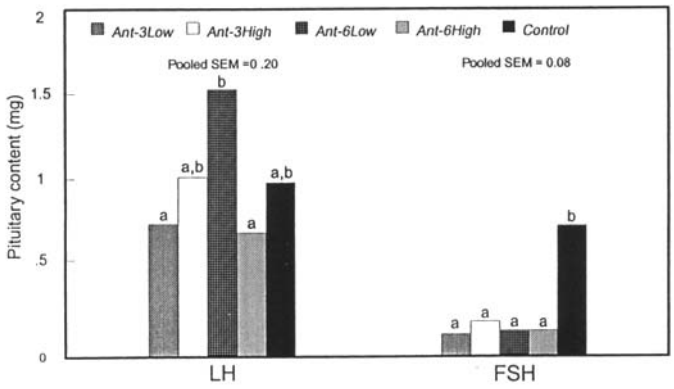

FIG. 2. Pituitary content (mg/pituitary) of LH and FSH. Ant-3Low and Ant-3High groups received 10 and 100 μg/kg of LHRH antagonist for 3 days, respectively. Ant-6Low and Ant-6High groups received 10 and 100 μg/kg of antagonist for 6 days, respectively. Control ewes received vehicle (1 ml of 5% mannitol). a,b) Bars with different letters differ ($p < 0.05$).

anterior pituitary tissue [26]. Treatment with SB-75 in the present study might have caused a down-regulation of LHRH receptors that further resulted in a decreased stimulation of gonadotrophs by endogenous LHRH. Consequently, changes in amounts of mRNA for gonadotropin subunits, content of pituitary gonadotropins, and concentrations of gonadotropins in circulation can be explained by changes in populations of LHRH receptors available to bind endogenous LHRH. Alternatively, LHRH receptors might have been present in the plasma membranes of gonadotrophs but not available to bind iodinated LHRH in the radioreceptor assay because they were occupied by antagonist.

Release of LH was reduced to less than 2 ng/ml approximately 8 h after treatment with LHRH-Ant, and remained low throughout the treatment period. As a result

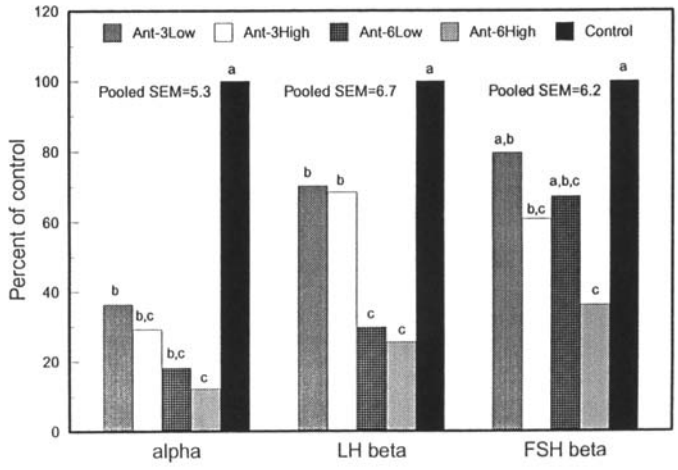

FIG. 3. Amounts of mRNA for α, LHβ, and FSHβ subunits. Values are expressed as percentage of controls. Ant-3Low and Ant-3High groups received 10 and 100 μg/kg of LHRH antagonist for 3 days, respectively. Ant-6Low and Ant-6High groups received 10 and 100 μg/kg of antagonist for 6 days, respectively. Control ewes received vehicle (1 ml of 5% mannitol). a,b,c) Bars with different letters differ ($p < 0.01$).

TABLE 2. Serum concentrations of LH and FSH (ng/ml) before and after treatment with LHRH-Ant.

Treatment	LH before[a]	LH after[b]	FSH before[a]	FSH after[b]
Control	14.7 ± 1.2	13.62 ± 3.13[c]	39.3 ± 9.0	40.0 ± 8.9[c]
Ant-3Low[e]	20.1 ± 6.8	0.35 ± 0.15[d]	28.9 ± 2.0	21.0 ± 1.2[d]
Ant-3High[e]	22.0 ± 2.5	0.20 ± 0.11[d]	37.3 ± 3.0	22.8 ± 3.7[d]
Ant-6Low[f]	26.7 ± 5.9	0.37 ± 0.13[d]	31.1 ± 2.0	17.0 ± 2.2[d]
Ant-6High[f]	12.4 ± 1.3	0.30 ± 0.09[d]	27.6 ± 3.0	13.8 ± 4.1[d]

[a]Samples were collected every 15 min for 4 h before initiation of treatments with LHRH-antagonist or vehicle (Mean ± SEM).
[b]Mean over a 10-h period (2-h samples starting 14 h after last treatment with LHRH-antagonist or vehicle).
[c,d]Different letters within the same column differ ($p < 0.01$).
[e]Low (10 μg/kg) or High (100 μg/kg) concentration of LHRH-antagonist every 24 h for 3 days.
[f]Low (10 μg/kg) or High (100 μg/kg) concentration of LHRH-antagonist every 24 h for 6 days.

of decreased LHRH stimulation of the gonadotrophs, suppressed release of LH was also reported in ovariectomized rats [5] and intact male rats [2, 28] in which LHRH was immunoneutralized with ovine LHRH antisera and [29] in men [30] and postmenopausal women [8] treated with an LHRH antagonist. Continued release of LH, therefore, appears to be dependent upon LHRH stimulation.

In contrast to LH, serum concentrations of FSH decreased only approximately 50% in the present study. A similar observation was reported in male rats after administration of LHRH antagonist [2, 5, 28], in rats in which LHRH was immunoneutralized with an ovine LHRH antiserum [29], and in men [30] and postmenopausal women treated with LHRH antagonist [8]. Basal release of FSH, therefore, appears to be only partially dependent upon stimulation by LHRH.

Pituitary content of LH did not differ among animals treated with LHRH-Ant compared to controls. In contrast, pituitary content of FSH was reduced in all ewes treated with LHRH. It is hypothesized that the decrease in pituitary stores of FSH resulted from continued release of FSH. In contrast, there was a 98% decrease in LH release, allowing maintenance of pituitary stores of LH. Apparently, endogenous LHRH in ewes from the Ant-6High group was maximally decreased and the ewes responded to LHRH-Ant treatment similarly to ewes with hypothalamic-pituitary disconnection [31].

A reduction in steady state amounts of mRNA for α and LHβ subunits occurred after treatment with LHRH-Ant in the present investigation. A similar decrease in amounts of α and LHβ subunits was observed previously in rats after treatment with an antagonist of LHRH [5] and in hypothalamic-pituitary-disconnected ewes [4]. Amounts of mRNA for FSHβ subunit differed in ewes treated with LHRH-Ant compared to control ewes but were not decreased to the same extent as α and LHβ subunits. The reduction in steady state amounts of mRNA for subunits of gonadotropins leads to the suggestion that gonadotroph function requires continued inputs from the hypothalamus. Previous work [32] also indicated that a pulsatile pattern of LHRH was needed to stimulate transcription of gonadotropin subunit genes.

It has been hypothesized that factors other than LHRH regulate the release of FSH [2–4, 33]. Chronic inhibition of pulsatile release of LHRH in intact ewes during the breeding season was reported to suppress amounts of FSHβ subunit mRNA and release of FSH by 30 to 50%, but amounts of LHβ subunit mRNA and LH release were suppressed by greater than 95% compared to control values [34]. Administration of LHRH antisera into the median eminence for 30 h failed to influence release of FSH but reduced release of LH [35]. A single treatment of anestrous ewes with ovine follicular fluid caused an initial suppression of FSH release that was not affected by the acute blockade of LHRH [36]. It was suggested that a component of FSH release is independent of LHRH but that the subsequent enhanced release of FSH that follows this initial suppression may have a component that is LHRH-dependent. The present experiment indicates that LHRH is necessary for synthesis of FSH in ewes. Release of FSH appeared to decline only in association with decreased stores of FSH in the pituitary and does not appear to be entirely dependent on LHRH. Our data support the hypothesis that differential regulation of LH and FSH release occurs in sheep. However, LHRH seems to be required for maintenance of steady state amounts of mRNA for the subunits of both pituitary gonadotropins.

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