REGULATION OF GENES CONTROLLING GONADOTROPIN SECRETION

Terry M. Nett

Colorado State University, Fort Collins 80523

ABSTRACT

Synthesis and secretion of gonadotropic hormones is a complex process that requires precise regulation of genes encoding for the gonadotropin-releasing hormone (GnRH) receptor, the common α-subunit of glycoprotein hormones and the individual β-subunits of the gonadotropins. These genes apparently can be influenced by gonadal steroids, GnRH and other peptide hormones such as activin and inhibin. Genes for the β-subunits of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) have been isolated and some information about their structures is available; however, at the time of this writing, the gene for the GnRH-receptor has not yet been isolated. Because elimination of hypothalamic input to the anterior pituitary gland is followed by a decrease in the number of GnRH-receptors and in the quantity of messenger RNA (mRNA) for the gonadotropin subunits, it is evident that a hypothalamic substance is required for normal expression of the genes for these proteins. This hypothalamic substance is probably GnRH, because pulsatile infusion of GnRH into animals without hypothalamic input to the anterior pituitary gland restores the number of GnRH-receptors and quantities of mRNA for the subunits of gonadotropins, as well as secretion of LH. Estradiol also appears to regulate genes for the GnRH-receptor and for the subunits of LH and FSH, but in an opposing manner in that it increases the number of receptors for GnRH but decreases the concentration of mRNA for the subunits of gonadotropins. Moreover, estradiol appears to be capable of overriding the stimulatory effect of GnRH on mRNA for the subunits of gonadotropins. In contrast to estradiol, progesterone appears to have little effect on expression of genes for the GnRH-receptor or for the gonadotropin subunits. Charcoal-treated bovine follicular fluid decreases the amount of mRNA for FSHβ-subunit presumably due to the inhibin it contains) but does not influence the amount of mRNA for α-subunit or LHβ-subunit. Concentrations of GnRH-receptors and mRNAs for gonadotropins subunits have been determined during the estrous cycle, gestation and postpartum anestrus. Changes in concentrations of mRNAs for gonadotropin subunits are discussed relative to the changes in concentrations of LH, FSH, estradiol and progesterone during these physiological states. Current concepts regarding our understanding of these events are depicted in Figure 1.

Introduction

The pituitary gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), are glycoprotein hormones composed of two dissimilar, non-covalently linked α- and β-subunits. Within a species the α-subunits are identical and are shared with thyroid-stimulating hormone and, if present, chorionic gonadotropin. In contrast, the β-subunits are dissimilar and appear to be responsible for conferring the respective biological properties of LH and FSH.

Synthesis of gonadotropins is a complicated process. The genes for the α- and β-subunits are located on separate chromosomes (Pierce, 1988) and thus are likely to be regulated independently of each other. Moreover, multi-
Figure 1. Schematic representation of our current understanding of the factors regulating synthesis and secretion of LH and FSH. When GnRH binds to its receptor on the membrane of a gonadotroph, it stimulates both the protein kinase C (PKC) and protein kinase A (PKA) second messenger systems, presumably by activating the stimulatory G protein (Gs). This, in turn, leads to activation of the genes encoding α- (not pictured), LHβ- and FSHβ-subunits. Inhibin likely binds to receptors on the membrane of gonadotrophs that may be coupled to an inhibitory G protein (Gi). This may lead to inhibition of the stimulatory activity of GnRH and activin on FSH synthesis and secretion. Because secretion (and possible synthesis) of LH is more sensitive to GnRH stimulation than FSH, the secretion and synthesis of LH continues even in the presence of inhibin. Interaction of estradiol with its receptor (E) inhibits transcription of the genes for both LHβ- and FSHβ-subunits. However, activation of the estradiol receptor increases the transcription of the gene encoding the receptor for GnRH (not pictured).
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FSH. Changes in these parameters that occur during different physiological states and due to experimental manipulation will be addressed. Because most studies of domestic animals in this area have utilized sheep as a model, this review will focus primarily on our current understanding of the regulation of gonadotropin genes in the ovine.

Structure of Genes

To date, the gene for the GnRH-receptor has not been isolated. However, the genes encoding α-subunit, LHβ-subunit and FSHβ-subunit have been isolated from a bovine genomic library and their sequences have been determined. The α-subunit gene contains 4 exons and 3 introns, and the coding region, including the 5' and 3' untranslated regions spans 16 kilobase pairs (Goodwin et al., 1983). In contrast, the LHβ-subunit gene is considerably smaller, spanning only 1.1 kilobase pairs; it contains 3 exons and 2 introns (Virgin et al., 1985). The FSHβ-subunit gene is intermediate in size, containing 4.0 kilobase pairs including 3 exons, 2 introns, a short 5' and a long 3' untranslated region (Kim et al., 1988). In addition to the coding regions, each of the genes for gonadotropin subunits also contains an upstream regulatory region responsible for controlling their level of expression. Unfortunately, only limited information is available regarding the structure of, or the specific factors that influence, the regulatory regions of gonadotropin genes in domestic animals. The human α-subunit gene contains a cyclic-AMP response element (CRE) that increases the rate of gene transcription when either the protein kinase A or protein kinase C second messenger system is activated (Andersen et al., 1988). The CRE is located approximately 110 bases upstream from the transcription start site on the gene (Bokar et al., 1988). A slightly modified CRE is also present on the bovine α-subunit gene in approximately the same location. Transcription of the rat LHβ-subunit gene also increases when the protein kinase C second messenger system is activated (Andrews et al., 1988); however, the location of the putative CRE on the regulatory region has not been identified. The human α-subunit gene also contains a tissue-specific element (TSE, a site on the gene that dictates that expression occur only in specific tissues) located approximately 210 bases upstream from the transcription start site (Windle et al., 1990). Examination of the nucleotide sequences of bovine genes coding for the gonadotropin subunits indicates that a sequence similar to the TSE identified on the human α-subunit gene is present at approximately the same location on each of the bovine gonadotropin subunit genes as the TSE on the human α-subunit gene. In addition to the CRE and TSE, an estrogen response element (ERE) has been identified on the rat LHβ-subunit gene approximately 1.2 kilobases upstream from the transcription start site (Shupnik et al., 1989). The ERE is a site on the regulatory region of the LHβ-subunit gene to which an activated estrogen receptor binds, thereby influencing the activity of the gene. In the rat, when the estrogen receptor binds to the ERE on the LHβ-subunit gene the rate of transcription is increased (Shupnik et al., 1989). Although not yet rigorously identified, it seems likely that genes encoding for the β-subunits of both LH (Nilson et al., 1983) and FSH (Kim et al., 1988) in domestic species also contain an ERE. Furthermore, it appears that when estrogen receptor binds to the ERE of these genes in sheep, transcription of the genes is inhibited (Nilson et al., 1983; Hamernik and Nett, 1988a). In contrast to the genes for the β-subunits of LH and FSH, there does not appear to be an ERE on the gene for the common α-subunit (Nilson et al., personal communication). Because the gene for α-subunit is also active in thyrotrophs and because production of thyroid-stimulating hormone is not known to be affected by estrogen, the lack of an ERE on the gene for α-subunit was not unexpected. A specific response element for progesterone receptor on genes for the gonadotropin subunits has not yet been identified.

Regulation of GnRH-receptors

Interaction of GnRH with its receptor on the plasma membrane of gonadotrophs represents the first step in the initiation of synthesis and secretion of LH and, to a lesser extent, FSH. The number of GnRH-receptors, as well as the amount of GnRH in the circulation, is important in determining the extent of gonadotroph activation (Wise et al., 1984). Therefore, it is important to consider factors that regulate the number of pituitary GnRH-receptors when considering regulation of gonadotropin synthe-
sis and secretion. During the estrous cycle, there is an increase in the number of GnRH-receptors in the anterior pituitary gland just prior to the preovulatory surge of gonadotropins with a decrease in the number of GnRH-receptors occurring by the end of the preovulatory surge (Crowder and Nett, 1984), probably due to down-regulation of receptors resulting from the increased secretion of GnRH during the surge itself (Moenter et al., 1989). After the preovulatory surge of gonadotropins there is an increase in the number of GnRH-receptors that occurs prior to the onset of luteal function (Figure 2). By the midluteal phase of the estrous cycle, the number of receptors for GnRH has returned to baseline and remains low for the remainder of the cycle.

Changes that occur in the concentration of GnRH-receptors during the estrous cycle of sheep suggest that regulation of these receptors is under hormonal control. In fact, GnRH itself and gonadal steroids appear to be major regulatory agents controlling the number of GnRH-receptors. As previously indicated, there is an increase in the number of GnRH-receptors in the anterior pituitary gland during the follicular phase of the estrous cycle. There is also an increase in the frequency at which pulses of GnRH are secreted into the portal vasculature during this phase (Clarke et al., 1987), leading to more pulses of LH, which, in turn, stimulates growth of the dominant follicle and increased secretion of estradiol. GnRH has been reported to increase the number of its own receptors (Nett et al., 1981; Conn et al., 1984). Likewise, estradiol increases the sensitivity of the anterior pituitary gland to GnRH (Reeves et al., 1971), presumably by stimulating an increase in the number of receptors for GnRH (Moss et al., 1981). Because estradiol also increases secretion of GnRH, it is difficult to determine whether the increase in GnRH-receptors that occurs after treatment with estradiol is due to a direct effect of estradiol on the anterior pituitary gland or is mediated via increased secretion of GnRH. To determine whether estradiol increases GnRH-receptors by acting directly at the anterior pituitary gland, we administered estradiol (25 μg, i.m.) to ovariectomized ewes in which the pituitary gland was surgically disconnected from the hypothalamus (hypothalamic-pituitary disconnection, HPD) using the procedure described by Clarke et al. (1983). The results from this experiment are depicted in Figures 3 and 4 (Gregg and Nett, 1989). There are several interesting points that can be derived from the data. First, HPD decreased basal secretion of LH and prevented the preovulatory-like surge of LH observed after administration of estradiol to hypothalamic-pituitary intact ewes. Second, the number of GnRH-receptors present after HPD was less than that in control ewes, indicating that secretion of GnRH is required to maintain a normal complement of GnRH-receptors. Third, there was an increase in the number of GnRH-receptors in HPD ewes after treatment with estradiol, proving that estradiol can act directly on the pituitary to stimulate an increase in the number of GnRH-receptors. Fourth, there was an increase in the number of GnRH-receptors in HPD ewes treated with estradiol without a concomitant increase in secretion of LH. This refutes the hypothesis that secretion of LH is required for incorporation of GnRH-receptors into the plasma membrane (Stemberger and Petrali, 1975).

Recently, we have undertaken studies to determine whether the increase in GnRH-receptors induced by estradiol is mediated via mechanisms requiring RNA and protein synthesis. For these studies, we utilized ovine anterior pituitary cells in culture. Treatment of these cells with estradiol (100 pg/ml) increased the number of GnRH-receptors by approximately threefold; however, if the cells were incubated in the presence of actinomycin D or cycloheximide, the increase in the number of GnRH-receptors normally induced by estradiol was prevented (Gregg et al., 1989). Collectively, these data indicate that estradiol induces an increase in pituitary GnRH-receptors via a classic genomic mechanism.

Changes in Gonadotropin mRNAs

Changes During the Estrous Cycle. Changes in circulating concentrations of gonadotropins during the ovine estrous cycle have been well documented (Niswender et al., 1976; Hauger et al., 1977). Recently, changes in the amount of mRNA for gonadotropin subunits in the anterior pituitary glands of sheep during the estrous cycle have also been reported (Leung et al., 1988; Herring et al., unpublished data). In general, at the onset of estrus the concentration of mRNA for LHβ-subunit is high but decreases by the end of the
Figure 2. Concentration of GnRH-receptors throughout the estrous cycle of ewes. Each bar represents the mean concentration of GnRH-receptors in the anterior pituitary glands of five ewes. "Pre" refers to tissue collected prior to the LH surge, and "Post" refers to tissue collected after the LH surge.

Figure 3. Serum concentrations of LH in ovariectomized ewes with an intact hypothalamic-pituitary axis (INT) or that had been subjected to hypothalamic-pituitary disconnection (HPD). Five INT and 5 HPD ewes were given 25 µg estradiol (E2) i.m., and an additional five ewes in each group were given an injection of vehicle (OIL) at 0 h. Data depicted are mean ± SEM of samples collected at hourly intervals (from Gregg and Nett, 1989).
Figure 4. Concentration of GnRH-receptors in the anterior pituitary glands of ovariectomized ewes with an intact hypothalamic-pituitary axis (INT) or that had been subjected to hypothalamic-pituitary disconnection (HPD). Five ewes in each group were given 25 μg estradiol (E2) i.m., and an additional five ewes were administered vehicle (OIL). Anterior pituitary glands were collected 16 h after administration of either E2 or OIL (from Gregg and Nett, 1989).

The amount of mRNA for LHβ-subunit increases during the remainder of diestrous. The content of LH in the anterior pituitary gland is high at the onset of estrus, decreases by ~85% by the end of the preovulatory surge and gradually returns to pre-surge levels by d 6 of diestrous, after which time it remains elevated throughout diestrous. Similar changes in pituitary content of LH were observed during the estrous cycle in cows (Nett et al., 1987).

Changes in the amount of mRNA for FSHβ-subunit did not occur in parallel to those described for LHβ-subunit. In fact, the amount of mRNA for FSHβ-subunit was low at the onset of estrus, increased gradually through d 6 of diestrous and then after d 10 of diestrous decreased until the onset of the next estrus (Figure 6). Changes in pituitary content of FSH appeared to follow changes in mRNA for FSHβ-subunit by approximately 4 d. Because changes in the amount of mRNA for FSHβ-subunit did not occur at the same time as changes in mRNA for LHβ-subunit, it appears that synthesis of mRNAs for the gonadotropin subunits is differentially regulated during the ovine estrous cycle. This differential regulation could occur via differential effects of gonadal steroids and inhibin on expression of the genes for gonadotropin subunits, or on stability of the messages. Because secretion of FSH, as well as the amount of mRNA for FSHβ-subunit, decreases during late diestrous and early estrus, it seemed likely that follicular inhibin (and estradiol) might be inhibiting expression of the FSHβ-subunit gene as well as decreasing secretion of FSH. To examine the possibility that inhibin may be responsible for this inhibition, charcoal-treated bovine follicular fluid (bFF) was administered to ewes for 7 d and then amounts of mRNA for

Figure 5. Amounts of mRNA for LHβ-subunit (relative units, R.U.) and concentration of LH in anterior pituitary glands from ewes at different stages of the estrous cycle. "Pre" refers to tissue obtained prior to the LH surge, and "Post" refers to tissue collected after the LH surge (n = 5/group).
gonadotropin subunits, growth hormone and prolactin in the anterior pituitary glands were quantified. Serum concentrations of FSH decreased (P < .05) by 76% in ewes treated with bFF. In contrast, serum concentrations of LH were unaffected by treatment (Figure 7, upper panel). The amount of mRNA for FSHβ-subunit fell to nondetectable levels after treatment, whereas the amounts of mRNA for α-subunit, LHβ-subunit, growth hormone and prolactin were not significantly affected by treatment (Figure 7, lower panel). Mercer et al. (1987) observed a rapid decrease (76% within 6 h) in the amount of mRNA for FSHβ-subunit in ovine anterior pituitary gland after administration of charcoal-treated bovine follicular fluid. Similar data have been reported for rats (Attardi et al., 1989). Thus, secretion of inhibin by developing follicles during late diestrus and early estrus may partially account for the differential changes in production of mRNAs for β-subunits of gonadotropins noted during these periods.

Changes During Anestrus. During her lifetime a female may experience several different types of anestrus. These include the anestrus prior to puberty (Foster et al., 1986), seasonal anestrus (Karsch et al., 1983), postpartum or lactational anestrus (Nett, 1987), nutritional anestrus (Gutierrez et al., 1987; Richards et al., 1989), or anestrus induced in response to many different kinds of stress (Gindoff and Ferin, 1987). The common factor associated with each of these types of anestrus is the lack of secretion of sufficient LH to stimulate follicular maturation. This appears to be the result of insufficient expression of genes encoding for subunits of LH. As a result, too little LH is synthesized to replace that which is secreted, leading to a decrease in pituitary content of this gonadotropin, and finally to an inability of the pituitary to secrete enough LH to induce follicular maturation (Moss et al., 1980; Nett, 1983). It should be noted that whereas too little circulating LH is common to each type of anestrus, the neural changes leading to the lack of LH secretion are probably quite different for each category of anestrus. It is beyond the scope of this review to consider each of these categories of anestrus in detail. Therefore, changes that occur during the anestrus associated with pregnancy and the postpartum period will be described to provide a generalized perception of the events occurring at the level of the anterior pituitary gland.

During pregnancy there is a dramatic reduction in the amount of LH contained in the anterior pituitary glands of sheep (Moss et al., 1980) and cattle (Moss et al., 1985; Nett et al., 1988). Pituitary content of LH is gradually replenished during the postpartum period, an event that must be completed before sufficient secretion of LH to initiate the final stages of follicular maturation can occur. We undertook

![Figure 6. Amounts of mRNA for FSHβ-subunit and concentration of FSH in anterior pituitary glands from ewes at different stages of the estrous cycle. "Pre" refers to tissue obtained prior to the preovulatory surge of gonadotropins, and "Post" refers to tissue collected after the preovulatory surge (n = 5/group).](image-url)
Figure 7. Serum concentrations of FSH and LH (upper panel) and amounts of mRNA for FSHβ-subunit, LHβ-subunit, α-subunit, GH and PRL (lower panel) in the anterior pituitary gland of ovariectomized ewes administered charcoal-treated bovine follicular fluid (treated) or saline (control) at 12-h intervals for 7 d. The mRNA for FSHβ-subunit was nondetectable (ND) in the treated animals (n = 5/group).

A series of studies to define the temporal relationship between changes in amounts of mRNA for gonadotropin subunits and pituitary content of gonadotropins during the postpartum period of ewes. We induced ewes to cycle and bred them during anestrus so that lambing would occur during the breeding season. Using this model we observed that the amount of mRNA for α-subunit fell by -85% during gestation, whereas the mRNA for LHβ-subunit decreased by more than 98% compared to levels observed during the midluteal phase of the estrous cycle (Wise et al., 1985). The comparatively lesser decrease in mRNA for α-subunit is presumably due to that present in thyrotrophs, which is probably not influenced by hormones secreted during pregnancy. The low levels of mRNA for α- and LHβ-subunit were associated with a >95% decrease in the amount of LH in the anterior pituitary gland by the end of gestation. Within 2 d after parturition, the amount of mRNA for α-subunit had increased approximately fourfold, whereas the amount of mRNA for LHβ-subunit had increased at least 10-fold compared to levels observed just prior to the end of gestation (Figure 8, lower panel). Maximal amounts of mRNA for the subunits of LH were observed by d 13 after parturition, and these levels were maintained through d 35. Even though amounts of mRNA for the subunits of LH had increased by 2 d after parturition, there was no detectable increase in pituitary content of LH at this time. In fact,
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Pituitary content of LH had only increased by ~50% on d 13 postpartum, when mRNAs for the subunits of LH had reached their maximum levels. It was not until d 22 postpartum that pituitary content of LH reached levels similar to those observed during the estrous cycle (Figure 8, upper panel). Thus, the increase in pituitary content of LH appears to require a period of several days after the increase in mRNA for α and LHβ-subunit. One possible reason for the relatively slow increase in pituitary content of LH after parturition is that the cellular machinery needed to synthesize and package LH is also deficient. In fact, the percentage of gonadotrophs in the anterior pituitary appears to double between d 2 and 35 after parturition. Besides increasing in number, the volume of individual gonadotrophs increases by nearly 50%. Moreover, the volume of the cell occupied by secretory granules increases by ~2.5-fold during this same interval (Wise et al., 1986). From these observations, it seems obvious that gonadotrophs are doing much more than making LH during the early postpartum period. That is, they are making cellular components for replication and secretory granules for storage of hormone and are increasing in size. Thus, it is not surprising that gonadotrophs are not particularly efficient at synthesizing LH during the early postpartum period.

The frequency and amplitude of LH pulses in the peripheral circulation of cows increased concomitantly with pituitary content of LH (Nett et al., 1988). Moreover, during the early postpartum period in ewes, there are many pulses of GnRH that are not accompanied by secretion of LH (Wise et al., 1989). As the period from parturition increases, a greater percentage of GnRH pulses lead to pulses of LH. Because the number of GnRH-receptors does not change with time after parturition (Crowder et al., 1982), it is tempting to speculate that the increased coincidence of GnRH and LH pulses is due to a greater releasable pool of LH in the anterior pituitary gland.

Requirement for Hypothalamic Input. Several different hormones influence secretion (and presumably synthesis) of gonadotropins. Whether these hormones act directly at the level of the anterior pituitary gland or their actions are mediated via alterations in the secretion of GnRH is less clear. We have utilized HPD to determine whether hypothalamic input is required to maintain pituitary content of mRNAs for subunits of the gonadotropins, prolactin and growth hormone, as well as pituitary content and secretion of these hormones in ewes (Hamernik et al., 1986; Hamernik and Nett, 1988b). By 1 wk after HPD the amount of mRNA for α-subunit, LHβ-subunit and FSHβ-subunit had decreased by 69%, 86% and 61%, respectively (Figure 9, middle panel). This was associated with a 59% and 52% reduction in pituitary content of LH and FSH (Figure 9, upper panel). The contents of mRNA for growth hormone and prolactin were not significantly affected by HPD. Serum concentrations of LH and FSH decreased (P < .05) after HPD, whereas secretion of prolactin increased (Figure 9, lower panel). Serum concentrations of growth hormone were not affected (data not shown). There was also an 83% decrease in the number of GnRH-receptors and a 70% decrease in the number of estrogen receptors 1 wk after HPD (Figure 9,
upper panel). If ewes were administered GnRH (250 ng over a period of 6 min once every 2 h) beginning 2 d after HPD, mRNAs for gonadotropin subunits increased ($P < .05$) compared to ewes receiving infusions of saline. Amounts of mRNA for $\alpha$ and FSH$\beta$-subunit were similar to those observed in hypothalamic-pituitary intact ewes, whereas the quantity of mRNA for LH$\beta$-subunit was only partially restored. Treatment of the HPD ewes with GnRH restored pituitary content of LH, serum concentrations of LH and the number of GnRH-receptors to levels similar to those observed prior to HPD. Although the quantity of mRNA for FSH$\beta$-subunit was restored to levels observed prior to HPD, treatment with GnRH was not effective in restoring either pituitary content or serum concentrations of FSH. Likewise, the number of estrogen receptors was not restored in HPD ewes given GnRH. Thus, it appears that either GnRH is not involved in the regulation of estradiol receptors in gonadotrophs, or that most estradiol receptors in the pituitary gland are in cells other than gonadotrophs. Administration of GnRH did not influence amounts of mRNA for growth hormone or prolactin, or serum concentrations of these hormones.
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From these data, it is reasonable to conclude that hypothalamic input is required to maintain the content of mRNA for the subunits of gonadotropins, pituitary content of LH and FSH, serum concentrations of LH and FSH, and receptors for GnRH. Administration of GnRH restored each of the parameters examined for synthesis and secretion of LH to normal or near normal, but it was not capable of restoring pituitary content or serum concentrations of FSH to normal within 7 d at the dosage or type of administration used. Clarke et al. (1986) also found that serum concentrations of FSH were much less dependent on pulses of GnRH than were serum concentrations of LH. It appears that a basal amount of FSH synthesis and secretion is maintained in the ewe, even in the absence of hypothalamic input. In contrast, maintenance of mRNA for growth hormone and prolactin does not appear to be dependent on hypothalamic input in ewes. In the absence of hypothalamic input, serum concentrations of prolactin increased, presumably due to the absence of inhibition by dopamine.

Effects of Progesterone. Progesterone decreases serum concentrations of LH, presumably by reducing the frequency at which pulses of GnRH are released from the hypothalamus (Goodman and Karsch, 1980; Karsch et al., 1987); however, by itself, progesterone does not appear to influence serum concentrations of FSH in ewes (Moss et al., 1981; Hamernik et al., 1987). Because progesterone decreases frequency, but not amplitude of pulses of GnRH, and because GnRH is required for maintenance of normal amounts of mRNA for the gonadotropin subunits, we tested the hypothesis that progesterone might decrease amounts of mRNA for the gonadotropin subunits in the anterior pituitary glands of ewes. Progesterone was administered to ovariectomized ewes to obtain luteal phase concentrations in blood for 3 wk, and then amounts of mRNA for the subunits of the gonadotropins, growth hormone and prolactin, pituitary content of LH and FSH, and serum concentrations of LH and FSH were determined (Hamernik et al., 1987). Although serum concentrations of LH were reduced by >95% in ewes treated with progesterone compared to control ewes, none of the other parameters examined (serum concentrations of FSH, pituitary content of LH and FSH, and amounts of mRNA for α-, LHβ- and FSHβ-subunits, growth hormone or prolactin) were affected by the treatment. Because progesterone reduces the frequency of GnRH pulses from about one per h to about one every 6 h, it appears that a frequency of one pulse of GnRH every 6 h is sufficient to maintain high levels of mRNA for the gonadotropin subunits in the ovine anterior pituitary gland.

Effects of Estradiol. Estradiol has been shown to have both positive (Goding et al., 1969; Beck and Reeves, 1973; Nett et al., 1974) and negative (Legan et al., 1977; Moss et al., 1981) feedback effects on the secretion of gonadotropins in ewes. In general, positive feedback of estradiol is noted within 24 h of treatment; however, if treatment is continued for longer periods, the effects of estradiol appear to become inhibitory to the secretion of gonadotropins (Tamanini et al., 1986). Therefore, it is possible that estradiol may also have a biphasic effect on mRNA for the subunits of gonadotropins and, ultimately, on synthesis of gonadotropins. To examine this possibility, ovariectomized ewes were administered estradiol via subcutaneous Silastic implants at a level sufficient to induce a preovulatory-like surge of gonadotropins (~30 pg/ml) for 3 wk. Anterior pituitary glands were collected at 0, .5, 1, 2, 4, 8 and 16 d relative to initiation of treatment, and amounts of mRNA for α-, LHβ- and FSHβ-subunits and pituitary content of gonadotropins were determined.

There was a triphasic effect of estradiol on serum concentrations of LH and FSH in ovariectomized ewes (Figure 10, upper panel). During the first 12 h after inserting estradiol implants, there was a decrease in serum concentrations of both LH and FSH. A preovulatory-like surge of the gonadotropins occurred between 12 and 24 h after implantation of estradiol; however, after 24 h of treatment serum concentrations of both LH and FSH had fallen to levels lower than those observed prior to administration of estradiol and continued to decrease for the duration of the experiment. There was also a significant reduction in the content of mRNA for each of the gonadotropin subunits in the anterior pituitary within 12 h (Figure 10, middle panel). Between .5 and 2 d of treatment, there was an increase in the steady-state levels of mRNA for the gonadotropin subunits, at which time maximum levels of mRNA were observed. The content of mRNA for α-subunit increased approximately ninefold and LHβ-subunit increased approximately twofold compared to
pretreatment levels. In contrast, mRNA for FSHβ-subunit only returned to pretreatment levels. Pituitary content of LH and FSH had decreased \((P < .05)\) within .5 d and remained below pretreatment levels throughout the entire period of the study (Figure 10, lower panel).

These data reveal several interesting points concerning how estradiol influences secretion of gonadotropins. First, the initial response to administration of estradiol to ovariectomized ewes was a decrease in each of the parameters measured that related to synthesis and secre-
tion of gonadotropins. That is, there was a decrease in serum concentrations of LH and FSH within 1 h of initiation of treatment and a significant decrease in the amount of mRNA for the subunits of gonadotropins 12 h after the initiation of treatment (the first time point examined); there was also a significant decrease in pituitary content of LH and FSH within 12 h of the beginning of treatment, most likely associated with the preovulatory-like surge of gonadotropins. There appears to be a large increase in the secretion of GnRH coinciding with the surge of gonadotropins (Moenter et al., 1989). It is presumed that this increase in secretion of GnRH stimulated the increase in mRNAs for gonadotropin subunits observed 2 d after initiation of treatment. The reason for the much larger increase in mRNA for α-subunit compared to LHβ- and FSHβ-subunits at d 2 of treatment is unknown. However, because there is an ERE on the gene for LHβ-subunit (Shupnik et al., 1989) and possibly on the FSHβ-subunit (Kim et al., 1988), it is entirely possible that as long as activated estrogen receptors are present to interact with the ERE, the rate of transcription induced by GnRH may be decreased. Because there does not appear to be an ERE on the gene encoding for α-subunit, the rate of transcription of the α-subunit gene may not be decreased by high circulating concentrations of estradiol, assuming that adequate secretion of GnRH occurs. This scenario might explain the much greater increase in the amount of mRNA for α-subunit relative to increases in mRNAs for LHβ- and FSHβ-subunits following the preovulatory-like surge of gonadotropins. The reason for the lack of increase in pituitary content of gonadotropins in view of the increases in mRNA for gonadotropin subunits is also unknown. It is possible that the constant high concentrations of estradiol prevents translation of the messages for one or both of the subunits for the gonadotropins. Alternatively, it is possible that estradiol may also increase the rate at which mRNAs for the subunits of gonadotropins are degraded. To date, neither of these possibilities has been critically examined.

Because prolonged treatment with estradiol decreases secretion of GnRH (Karch et al., 1987), it is possible that the decrease in mRNAs for gonadotropin subunits could have been entirely due to effects mediated at the level of the hypothalamus. However, as indicated above, there is an ERE on the gene encoding for LHβ-subunit in rats (and likely in other species as well), and there is some indication that the FSHβ-subunit gene in sheep is also responsive to estradiol (Phillips et al., 1988). To test the hypothesis that estradiol can act directly at the level of the anterior pituitary gland to inhibit synthesis of LH, we conducted the following experiment. Ten ovariectomized ewes were treated with progesterone and estradiol for 80 d at levels similar to those observed during the last trimester of pregnancy to decrease pituitary content of LH. After 40 d of treatment, five of the ewes were administered pulses of GnRH (250 ng once every 2 h) for an additional 40 d. Five ovariectomized ewes not treated with steroid served as controls. At the end of the experiment, the pituitary glands were collected and analyzed for content of LH. The content of LH was 504 ± 122 µg/g in the anterior pituitary glands of the ovariectomized control ewes, 114 ± 6 µg/g in ewes treated only with progesterone and estradiol, and 56 ± 3 µg/g in ewes treated with progesterone and estradiol and given pulses of GnRH. Thus, progesterone and estradiol at concentrations similar to those that occur near the end of gestation effectively decreased pituitary content of LH. Because this reduction in pituitary content of LH induced by steroid treatment could not be overcome by administration of GnRH at a dosage shown to stimulate accumulation of LH in the anterior pituitary of ewes after HPD (Hamernik and Nett, 1988b), we suggest that estradiol inhibits synthesis of LH directly at the level of the anterior pituitary gland. The mechanism of this inhibition is as yet unknown but may involve suppression of gene transcription and/or reduced translational efficiency in gonadotrophs.

**Literature Cited**


