Gonadotrope responsiveness in orchidectomized sheep: effect of duration of a simulated follicular phase

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The effect of duration of a simulated follicular phase on gonadotrope responsiveness was assessed in orchidectomized sheep (wethers). The oestrogenic and hypothalamic inputs characteristic of the ovine follicular phase were simulated by continuous infusion of oestradiol ($5 \mu\text{g h}^{-1}$ in 10% (v/v) ethanol-saline) and circhoral delivery of GnRH (200 ng per hourly pulse) for 0, 6, 12, 24, 48 or 96 h ($n = 6$ wethers per group). Responsiveness increased ($P < 0.05$) with increasing duration of simulated follicular phase. In a second experiment, responsiveness was assessed 96 h after initiation of infusion of oestradiol in wethers receiving hourly pulses of GnRH or saline. Concurrent administration of GnRH reduced ($P < 0.05$) the magnitude of the oestradiol-induced increase in gonadotrope responsiveness. In a companion study, anterior pituitary tissue was collected 96 h after the start of infusion of oestradiol and circhoral delivery of GnRH or saline. Pituitary stores of LH and tissue concentrations of GnRH receptor and mRNA encoding the GnRH receptor were increased ($P < 0.05$) by oestradiol infusion. The magnitude of these oestradiol-induced responses was not affected ($P > 0.05$) by concurrent GnRH treatment. Tissue concentrations of FSH and mRNA encoding the FSH β subunit were decreased ($P < 0.05$) by oestradiol infusion. This suppressive effect of oestradiol was not reversed by GnRH. These results indicate that oestradiol stimulation, but not concurrent delivery of GnRH, is essential for full expression of surge-like secretion of LH. In addition, the oestradiol-induced increase in gonadotrope responsiveness during the simulated follicular phase is sustained throughout the period of oestradiol delivery.

Introduction

In previous studies we have used oestradiol-treated orchidectomized sheep (wethers) to characterize the pattern of GnRH stimulation required to induce preovulatory surge-like secretion of LH (Sakurai and Adams, 1991; Sakurai *et al.*, 1993a). The results of these studies suggest that surge-like secretion of LH is induced by an abrupt increase in the amplitude of the circhoral GnRH stimulus. This pattern of GnRH delivery is referred to as the 'stimulus-shift' paradigm. This regimen of GnRH or GnRH agonist delivery involves a period of low amplitude stimulation that simulates the pattern of GnRH secretion during the follicular phase (Moenter *et al.*, 1991). Ovulatory surge-like secretion of the gonadotrophins is induced by an eight-fold increase in the amplitude of the circhoral GnRH or GnRH agonist input. The physiological relevance of this paradigm has been demonstrated in female sheep in which endogenous GnRH has been neutralized by passive immunization (Sakurai *et al.*, 1992). Moreover Moenter *et al.* (1990, 1991) have characterized a pattern of GnRH secretion during the periovulatory period in ewes that is similar to the stimulus-shift paradigm of GnRH administration.

Oestrogenic stimulation during the simulated follicular phase is a critical determinant of gonadotrope responsiveness. Indeed, the surge-like secretion of LH induced in wethers and ewes at stimulus-shift is blunted in the absence of oestrogenic stimulation, or after oestradiol withdrawal (Sakurai *et al.*, 1992, 1993a, b). Oestradiol secretion during the follicular phase of the oestrous cycle is the product of an endocrine cascade that begins at the hypothalamus with episodic secretion of GnRH. In addition to sustaining gonadotrophin release and promoting oestradiol secretion from the developing Graafian follicle, low amplitude GnRH stimulation during the natural or simulated follicular phase may also directly affect gonadotrope responsiveness. Clarke and Cummins (1987) reported that episodic low-amplitude GnRH stimuli augmented gonadotrope responsiveness by supporting LH and FSH synthesis and maintaining tissue stores of these hormones. Indeed, pulsatile delivery of GnRH increases the concentration of mRNA encoding the gonadotrophin subunits in pituitary tissue and augments tissue concentrations of GnRH receptor (Leung *et al.*, 1987; Hamernik and Nett, 1988). Furthermore, Phillips *et al.* (1990) suggested that low-amplitude GnRH stimulation during the follicular phase plays a key role in determining the magnitude of the secretory response induced by an abrupt increase in the amplitude of the GnRH stimulus. Taken together, these studies suggest that the augmentation of gonadotrope responsiveness

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that is essential for full expression of the GnRH-induced preovulatory surge of gonadotrophins is the product of oestrogenic stimulation and circhoral stimulation by low amplitude GnRH.

In the study reported here the relative contributions of oestradiol and low amplitude GnRH stimulation to the enhancement of gonadotrope responsiveness that occurs during the simulated follicular phase is examined. We hypothesized that pulsatile delivery of low amplitude GnRH would increase gonadotrope responsiveness in oestradiol-treated wethers. In addition, we postulated that increasing the duration of the simulated follicular phase would lead to corresponding increases in gonadotrope responsiveness.

Materials and Methods

Animals

Crossbred whiteface sheep were castrated within 2 weeks of birth. The orchidectomized lambs (wethers) were housed in an open-sided barn under natural lighting and were afforded free access to water and alfalfa pellets supplemented with cereal grains and vitamin and mineral premix. The studies described here were conducted in early spring, a period of transition between the breeding and anoestrous seasons in female sheep at this latitude (38°N). The wethers were 6–8 months of age (mass = 45–50 kg) at the time of experimentation. All experimental procedures involving the use of animals were conducted in accordance with National Institutes of Health (NIH) Guidelines and were reviewed and approved by the Animal Use and Care Committee for the University of California.

Cannulation

Before experimentation, two polyethylene cannulae (Intramedic PE 100, Clay Adams, Parsippany, NJ) were inserted into the left jugular vein to serve as hormone delivery cannulae (oestradiol or GnRH). A third cannula (Intramedic PE 190), inserted into the contralateral vein, was used for collection of blood samples. All cannulae were passed through a protective Tygon tubing sheath to the exterior of the animal holding area. Animals were freely mobile at the end of a 1 m lead.

Hormone delivery

Cannulae for the delivery of oestradiol or vehicle were connected to syringes placed in Harvard infusion pumps (Model 2265, Harvard Bioscience, South Natick, MA). Oestradiol ($5 \mu\text{g ml}^{-1}$; Sigma Chemical Co., St Louis, MO) in 10% (v/v) ethanol-saline (vehicle), or vehicle alone, was continuously infused at a rate of 1 ml h^{-1} . Serum concentrations of oestradiol were increased to $27.9 \pm 4.5 \text{ pg ml}^{-1}$ within 1 h of initiation of oestradiol infusion and remained at that concentration for the duration of oestradiol delivery. In contrast, serum concentrations of oestradiol were not detectable ($< 0.6 \text{ pg ml}^{-1}$) in wethers receiving vehicle alone.

An episodic pattern of delivery of GnRH (200 or 1600 ng per hourly pulse) in saline, or saline alone, was effected using an

infusion pump connected to an automatic timer (Chronotrol, Lindburg Enterprises, San Diego, CA) which was programmed to deliver a 5 min pulse (1 ml volume) once each hour. During episodic administration of GnRH, blood samples were collected immediately before GnRH delivery.

Experiment 1

Oestrogenic and hypothalamic inputs characteristic of the follicular phase were simulated in wethers by continuous infusion of oestradiol and circhoral delivery of low amplitude GnRH. The effect of duration of the simulated follicular phase on gonadotrope responsiveness was determined. Thirty-six wethers were separated at random into 6 treatment groups ($n = 6$ animals per group). Infusion of oestradiol and episodic delivery of GnRH (200 ng per hourly pulse) were continued for 6, 12, 24, 48 or 96 h in groups B–F, respectively. Gonadotrope responsiveness was assessed at the end of the period of low amplitude GnRH treatment by increasing the amplitude of the GnRH stimulus to 1600 ng per hourly pulse (stimulus-shift). High amplitude stimulation was continued for 24 h. Oestradiol infusion was maintained throughout the GnRH delivery period. In treatment group A, gonadotrope responsiveness was assessed in animals that had not been previously treated with either oestradiol or low amplitude GnRH. Blood samples were collected at intervals of 2 h during the first 12 h of the simulated follicular phase and at 6 h intervals thereafter. During high amplitude GnRH stimulation blood samples were collected at 2 h intervals. Serum was harvested and stored frozen at -20°C for later endocrine analysis.

Experiment 2

The effect of episodic delivery of low amplitude GnRH or continuous infusion of oestradiol or both factors on gonadotrope responsiveness was examined in another experiment. Eighteen wethers were divided into 3 groups ($n = 6$ wethers per group). Animals in groups 2A and 2B received oestradiol ($5 \mu\text{g h}^{-1}$) as a continuous infusion for 120 h. Circhoral delivery of saline (group 2A) or low amplitude GnRH (200 ng per hourly pulse, group 2B) was initiated at the beginning of oestradiol infusion and continued through the first 96 h of infusion. Gonadotrope responsiveness was assessed during the final 24 h of infusion by episodic delivery of a high amplitude stimulus (1600 ng GnRH per hourly pulse). Control animals (group 2C) were infused with the oestradiol delivery vehicle and received hourly pulses of saline during the 96 h period before stimulus-shift. Blood samples were collected at intervals of 2 h during the first 12 h of the 96 h simulated follicular phase and at intervals of 6 h thereafter. During high amplitude GnRH stimulation blood samples were collected at intervals of 2 h. Serum was harvested and stored frozen at -20°C for later hormone analysis.

Experiment 3

The effect of episodic delivery of low amplitude GnRH or continuous infusion of oestradiol or both factors on anterior pituitary function was assessed in a companion study. Eighteen

Fig. 1. Gonadotrope responsiveness in wethers ($n = 6$ animals per group) 0 ($\circ$), 6 ($\bullet$), 12 (Δ), 24 ($\blacktriangle$), 48 ($\diamond$), or 96 ($\blacklozenge$) h after the start of continuous infusion of oestradiol ($5 \mu\text{g h}^{-1}$) and concurrent episodic delivery of low amplitude GnRH (200 ng per hourly pulse). The increase in serum concentration of LH, above basal, during the first 12 h of circhoral delivery of high amplitude GnRH (1600 ng per hourly pulse) was taken as a measure of gonadotrope responsiveness. Error bars have been omitted for clarity.

wethers were divided into 3 groups ($n = 6$ wethers per group). Animals in groups 3A and 3B received oestradiol ($5 \mu\text{g h}^{-1}$) as a continuous infusion for 96 h. Circhoral delivery of saline (group 3A) or low amplitude GnRH (200 ng per hourly pulse, group 3B) was continued throughout the period of oestradiol infusion. Control animals (group 3C) were infused with the oestradiol delivery vehicle and received hourly pulses of saline for 96 h. At the conclusion of the 96 h infusion period, animals were stunned by means of a captive bolt pistol and killed by exsanguination at the UC Davis Slaughter Facility. Anterior pituitary tissue was quickly excised, halved by a midsagittal cut and each half was immediately frozen in liquid nitrogen and stored at -70°C for later analysis.

Endocrine analysis

Serum and tissue concentrations of LH and FSH, and serum concentrations of oestradiol were determined using previously validated procedures (Adams *et al.*, 1975, 1988; Sakurai *et al.*, 1992). The LH and FSH reference standards (NIAMDD-oLH-23 and NIAMDD RP-1) were gifts from the National Hormone and Pituitary Program of the National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD; Baltimore, MD). In all cases intra- and inter-assay coefficients of variation were less than 10%.

The affinity and tissue concentration of GnRH receptors were quantified by means of the procedure described previously (Sakurai and Adams, 1991). Tissue concentrations of mRNA encoding the GnRH receptor or the gonadotrophin subunits were determined via the solution hybridization procedures described previously (Sakurai *et al.*, 1993b; Adams *et al.*, 1996). Plasmids containing cDNA inserts for the bovine α (Erwin *et al.*, 1983), LH β (Maurer, 1985) and FSH β (Maurer and Beck, 1986) subunits were kindly provided by R. Maurer

(Department of Cell Biology and Anatomy, Oregon Health Sciences University, Portland, OR). A plasmid containing the cDNA insert for the ovine GnRH receptor (Brooks *et al.*, 1993) was kindly provided by J. Brooks (MRC Reproductive Biology Unit, Edinburgh). The sense and anti-sense cRNAs were generated from linearized cDNA by transcription *in vitro* using either T7 or SP6 RNA polymerase and the Riboprobe Gemini System II reagent system (Promega Corp., Madison, WI).

Statistical analyses

The significance of treatments was assessed by analysis of variance (Gill, 1978). Differences between treatment means were tested for significance using Duncan's multiple range test. During assessment of gonadotrope responsiveness high amplitude GnRH stimulation was continued for 24 h. However, pretreatment concentrations of LH and FSH were re-established 12 and 6 h after stimulus-shift, respectively. Therefore, the total amount of LH or FSH released during the first 12 h or 6 h, respectively, after stimulus-shift was taken as a measure of gonadotrope responsiveness.

Results

Experiment 1

Gonadotrope responsiveness was significantly ($P < 0.05$) increased within 6 h of initiation of oestradiol infusion and circhoral delivery of low amplitude GnRH (Fig. 1 and Table 1). Extending the duration of treatment resulted in further increases in the magnitude of LH secretion during high amplitude GnRH stimulation. Continuous infusion of oestradiol

Table 1. Effect of duration of a simulated follicular phase on the magnitude of gonadotrophin secretion induced by a circhoral high amplitude GnRH stimulus in orchidectomized sheep (wethers)

Duration of simulated follicular phase (h) ^a	LH ^b (ng ml ⁻¹ (12 h) ⁻¹)	FSH ^b (ng ml ⁻¹ (6 h) ⁻¹)
0	57.8 ± 14.5 ^c	0.7 ± 0.1 ^c
6	193.4 ± 10.5 ^d	7.5 ± 1.0 ^d
12	236.0 ± 36.2 ^d	9.9 ± 1.5 ^d
24	249.2 ± 25.9 ^d	8.9 ± 0.8 ^d
48	278.3 ± 17.5 ^d	8.8 ± 0.7 ^d
96	396.5 ± 63.6 ^c	8.2 ± 1.4 ^d

^aA simulated follicular phase was established in wethers by continuous infusion of oestradiol (5 µg h⁻¹) and episodic delivery of low amplitude GnRH (200 ng per hourly pulse) for 0, 6, 12, 24, 48, or 96 h.

^bArea under the secretory profile of LH and FSH, in excess of basal secretion, during circhoral delivery of high amplitude GnRH (1600 ng per hourly pulse). The total amount of LH or FSH released during the first 12 h or 6 h, respectively, after stimulus-shift was taken as a measure of gonadotrope responsiveness. Values are presented as means ± SEM (*n* = 6 animals per group).

^cValues in a column that do not share a common superscript are significantly different (*P* < 0.05).

and episodic administration of low amplitude GnRH resulted in a tenfold increase (*P* < 0.05) in the magnitude of FSH secretion induced at stimulus-shift (Table 1). In contrast to the secretory response of LH, the magnitude of high amplitude GnRH-induced FSH secretion did not vary with duration of simulated follicular phase.

Experiment 2

Serum concentrations of LH (13.4 ± 1.8 ng ml⁻¹) and FSH (14.9 ± 0.7 ng ml⁻¹) in control wethers infused with the oestradiol delivery vehicle and receiving hourly pulses of saline for 96 h did not differ (*P* > 0.05) from pretreatment values (13.6 ± 1.2 ng ml⁻¹ and 15.1 ± 0.6 ng ml⁻¹ for LH and FSH, respectively). In contrast, serum concentrations of LH and FSH were decreased (*P* < 0.05) in wethers receiving oestradiol and hourly pulses of saline (Fig. 2). Serum concentrations of LH were decreased by 50% (6.6 ± 0.8 ng ml⁻¹) within 8 h of initiation of infusion and remained at that concentration for the duration of oestradiol delivery. Serum concentrations of FSH were significantly reduced within 4 h of initiation of infusion and continued to decrease gradually throughout the remainder of oestradiol delivery. The magnitude of the oestradiol-induced decrease in serum concentrations of FSH was not affected (*P* > 0.05) by episodic delivery of low amplitude GnRH. In contrast, the pattern of LH secretion during oestradiol infusion and concurrent delivery of low amplitude GnRH (200 ng per hourly pulse) was biphasic. Serum concentrations of LH were significantly decreased during the early period of combined oestradiol and GnRH treatment, with serum concentrations of LH reaching a nadir (6.3 ± 0.7 ng ml⁻¹) 10 h after initiation of the combined treatment. Serum concentrations of LH increased thereafter and returned to pretreatment concentrations 36 h after initiation of oestradiol infusion and circhoral administration of low amplitude GnRH (Fig. 2).

Fig. 2. Serum concentrations of (a) LH and (b) FSH in wethers (*n* = 6 animals per group) during continuous infusion of oestradiol (5 µg h⁻¹) and concurrent episodic delivery of saline (○) or low amplitude GnRH (200 ng per hourly pulse; ●). Control wethers (△) received oestradiol delivery vehicle and hourly pulses of saline during the 96 h infusion period.

High amplitude GnRH stimulation (stimulus-shift) induced surge-like secretion of LH in wethers receiving continuous infusion of oestradiol for 96 h (Fig. 3). In contrast, high amplitude GnRH stimulation was unable to elicit surge-like release of LH in wethers infused with vehicle alone. The magnitude of LH release in response to stimulus-shift in wethers receiving oestradiol infusion and low-amplitude GnRH (200 ng per hourly pulse) stimulation was significantly (*P* < 0.05) reduced, relative to the LH response in wethers receiving oestradiol alone during the 96 h before stimulus-shift (Fig. 3).

Experiment 3

Oestradiol infusion alone or in combination with circhoral delivery of GnRH resulted in a fourfold increase in tissue concentrations of GnRH receptor and mRNA encoding the GnRH receptor (Fig. 4). Similarly, the concentration of LH in the pituitary tissue of wethers receiving oestradiol or the oestradiol and GnRH combination for 96 h was significantly greater than the tissue concentration of LH in control animals (Fig. 5). The magnitude of the oestradiol-induced augmentation of tissue concentrations of GnRH receptor or LH stores was not significantly affected by concurrent delivery of low amplitude GnRH pulses. In contrast, tissue concentrations of FSH and steady state amounts of mRNA encoding the FSHβ subunit

Fig. 3. Serum concentrations of LH in wethers during circhoral delivery of high amplitude GnRH (1600 ng per hourly pulse; stimulus-shift). Wethers ($n = 6$ animals per group) received oestradiol ($5 \mu\text{g h}^{-1}$) and hourly pulses of saline (○) or low amplitude GnRH (200 ng per hourly pulse; ●) during the 96 h period preceding stimulus-shift. Control wethers (△) were infused with oestradiol delivery vehicle and received hourly pulses of saline during the 96 h period preceding stimulus-shift.

were significantly ($P < 0.05$) decreased after 96 h of oestradiol infusion (Fig. 6). The reduction in FSH and mRNA for the FSH β subunit was not affected ($P > 0.05$) by concurrent delivery of low amplitude GnRH. Neither oestradiol nor the oestradiol and low amplitude GnRH combination had a significant effect on steady state amounts of mRNA for the LH β (Fig. 5). Similarly, the tissue concentration of mRNA encoding the α subunit in wethers receiving oestradiol ($41.7 \pm 5.3 \text{ pg } \mu\text{g}^{-1} \text{ total RNA}$) or the oestradiol and GnRH combination ($34.1 \pm 7.3 \text{ pg } \mu\text{g}^{-1} \text{ total RNA}$) for 96 h did not differ ($P > 0.05$) from the amount of mRNA for the α subunit in pituitary tissue of control animals ($36.5 \pm 6.0 \text{ pg } \mu\text{g}^{-1} \text{ total RNA}$).

Discussion

We have previously demonstrated that an eightfold increase in the amplitude of hourly pulses of GnRH produces preovulatory

Fig. 4. Concentrations of (■) GnRH receptor (GnRH-R) and (▨) mRNA encoding the GnRH receptor in pituitary tissue of wethers ($n = 6$ animals per group) after 96 h of continuous infusion of oestradiol ($5 \mu\text{g h}^{-1}$) and concurrent episodic delivery of saline (E2/Sal) or low amplitude GnRH (200 ng per hourly pulse; E2/GnRH). Control wethers (Veh/Sal) were infused with oestradiol delivery vehicle and received hourly pulses of saline during the 96 h period preceding tissue collection. FTE: fresh tissue equivalent.

Fig. 5. Concentrations of (■) LH and (▨) mRNA for the LH β subunit in pituitary tissue of wethers ($n = 6$ animals per group) after 96 h of infusion of oestradiol ($5 \mu\text{g h}^{-1}$) and concurrent episodic delivery of saline (E2/Sal) or low amplitude GnRH (200 ng per hourly pulse; E2/GnRH). Control wethers (Veh/Sal) were infused with oestradiol delivery vehicle and received hourly pulses of saline during the 96 h period preceding tissue collection. FTE: fresh tissue equivalent.

surge-like secretion of LH in wethers receiving oestradiol (Sakurai *et al.*, 1993a). The physiological relevance of this 'stimulus-shift' pattern of GnRH delivery has been confirmed in female sheep passively immunized against GnRH (Sakurai *et al.*, 1992). Oestrogenic input appears to be critical for full expression of the GnRH-induced surge, since the stimulus-shift pattern of GnRH or GnRH agonist delivery is unable to effect surge-like secretion of LH in wethers or ewes in the absence of concurrent endogenous or exogenous oestradiol stimulation (Sakurai *et al.*, 1992, 1993a). Similarly, the magnitude of the GnRH-induced surge rapidly decays after withdrawal of oestrogenic support (Sakurai *et al.*, 1993b). The experiments reported here examine the effect of duration of oestrogenic exposure on the magnitude of the LH and FSH surges induced by stimulus-shift.

Fig. 6. Concentrations of (■) FSH and (▨) mRNA for the FSH β subunit in pituitary tissue of wethers ($n = 6$ animals per group) after 96 h of continuous infusion of oestradiol ($5 \mu\text{g h}^{-1}$) and concurrent episodic delivery of saline (E2/Sal) or low amplitude GnRH (200 ng per hourly pulse; E2/GnRH). Control wethers (Veh/Sal) were infused with oestradiol delivery vehicle and received hourly pulses of saline during the 96 h period preceding tissue collection. FTE: fresh tissue equivalent.

These studies demonstrate that the magnitude of secretion of LH induced at stimulus-shift increases with duration of oestrogenic exposure. This confirms and extends our previous observations (Sakurai *et al.*, 1993a) and is consistent with the response to extended oestrogenic stimulation noted in women (Keye and Jaffe, 1975) and cattle (Kesner *et al.*, 1981). However, these results are in contrast to the oestrogenic response reported using ovine pituitary cells in culture. Miller and co-workers (Miller and Huang, 1985; Laws *et al.*, 1990) noted that the responsiveness of gonadotrope cells was first increased, then markedly reduced, during extended (> 24 h) culture in oestradiol-containing media. This biphasic response to oestradiol stimulation noted *in vitro* has led to the suggestion that oestradiol stimulation plays a critical role in both the initiation and termination of the ovulatory surge of gonadotrophins (Laws *et al.*, 1990). The studies detailed here serve to emphasize the importance of oestradiol in initiation of the preovulatory surge. However, our results, using an *in vivo* model, do not support the contention that prolonged exposure to oestradiol leads to a diminution of gonadotrope responsiveness. Indeed, in our studies oestrogenic stimulation consistently enhanced gonadotrope responsiveness, regardless of duration of exposure. This finding suggests that the descending limb of the ovulatory surge is not driven by direct inhibitory effects of oestradiol. Rather, the reduction in gonadotrope responsiveness during the descending limb of the LH surge may reflect GnRH-induced depletion of pituitary stores of LH, downregulation of the GnRH receptor, or desensitization of a second messenger system or a combination of these effects (Sakurai *et al.*, 1993a; Adams *et al.*, 1996).

The direct contribution of low amplitude GnRH stimulation to augmentation of gonadotrope responsiveness during the natural or simulated follicular phase has not been clearly defined. Episodic delivery of GnRH is required to maintain gonadotrope function. Indeed, tissue concentrations of LH and FSH, steady state concentrations of mRNA coding for the gonadotrophin subunits, and tissue content of GnRH receptor are decreased after hypothalamo-pituitary disconnection (HPD;

Hamernik *et al.*, 1986), administration of a GnRH antagonist (Sanchez *et al.*, 1994) or immunoneutralization of GnRH (Sakurai and Adams, unpublished). Moreover, these measures of gonadotrope function are restored in HPD sheep by pulsatile delivery of GnRH (Hamernik and Nett, 1988). However, the studies reported here demonstrate that the magnitude of the LH surge induced at stimulus-shift is reduced in wethers receiving low amplitude GnRH stimulation. Circchoral administration of low amplitude GnRH during the simulated follicular phase may reduce the magnitude of the surge induced at stimulus-shift by mobilizing, and thus depleting, the LH in a readily releasable intracellular pool.

Although gonadotrope responsiveness was enhanced during oestradiol stimulation, gonadotrophin secretion was depressed during the period of oestradiol delivery. The oestradiol-induced decrease in gonadotrophin secretion is likely to reflect oestrogenic action at hypothalamic loci to reduce secretion of GnRH (Tilbrook *et al.*, 1991; Tilbrook and Clarke, 1995), since episodic delivery of exogenous GnRH restored LH secretion and serum concentrations of LH. It is likely that oestradiol-induced augmentation of gonadotrope responsiveness is due, at least in part, to replenishment of readily releasable pools of LH during oestradiol-dependent suppression of gonadotrophin secretion.

Continuous infusion of oestradiol for 96 h resulted in a marked increase in the concentration of GnRH receptor and mRNA encoding the GnRH receptor in pituitary tissue. This oestradiol-induced response in wethers is comparable to the change in tissue content of GnRH receptor and mRNA for the GnRH receptor noted in oestradiol-treated ovariectomized sheep (Turzillo *et al.*, 1994). The oestradiol-induced increase in tissue content of GnRH receptor and mRNA for the GnRH receptor noted in these studies is likely to reflect, at least in part, direct action of oestradiol at pituitary loci since the oestrogen-stimulated response is noted in sheep with HPD (Turzillo *et al.*, 1995). A similar oestrogenic response has also been noted in sheep in which hypothalamic inputs have been negated by passive immunization (Adams and Sakurai, unpublished) or prolonged administration of a GnRH antagonist (Brooks and McNeilly, 1994). Oestrogen-induced augmentation of GnRH receptor and mRNA for the GnRH receptor has also been noted *in vitro*, using ovine pituitary cells in culture (Sealfon *et al.*, 1990; Wu *et al.*, 1994).

Physiological concentrations of oestradiol markedly depressed tissue content of mRNA for the FSH β subunit, but had no effect on steady-state content of mRNA for the α and LH β subunits. This is consistent with previous studies (Sakurai *et al.*, 1993a, b). In the study reported here the suppressive effect of oestradiol on tissue content of mRNA for the FSH β subunit is not reversed by the concurrent delivery of low amplitude GnRH. A similar response to oestrogenic stimulation has been noted in HPD sheep receiving exogenous GnRH (Mercer *et al.*, 1988; 1989). These results indicate that oestradiol acts directly at hypophyseal loci to modulate FSH β gene transcription or the stability of mRNA for the FSH β subunit or both processes.

The results of these studies suggest that the magnitude of the gonadotrophin surge that is induced at stimulus-shift is primarily determined by the extent of oestrogenic stimulation. Increasing the duration of oestradiol stimulation appears to

enhance responsiveness and the magnitude of the surge. Pulses of low amplitude GnRH during the simulated follicular phase lessen the magnitude of surge-like secretion induced by stimulus-shift, perhaps by reducing the LH resident in a readily releasable pool. However, circhoral low amplitude GnRH stimulation is likely to play a critical physiological role during the periovulatory period, since this pattern of GnRH stimulation is required to sustain gonadotrophin secretion adequate to promote follicular growth and development and oestradiol secretion.

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