To test the hypothesis that low levels of estradiol are sufficient to increase concentrations of GnRH receptor mRNA in the absence of progesterone, ewes were ovariectomized and immediately treated with estradiol implants for 12 h to achieve circulating concentrations of estradiol typical of the early (n = 5) or late (n = 4) follicular phase. Five additional ewes underwent luteectomy and control ewes were untreated. Treatment of ewes with ½ or 1 estradiol implant increased concentrations of estradiol in serum to 3.0 ± 0.8 pg/ml or 6.3 ± 0.3 pg/ml, respectively, and concentrations of estradiol in luteectomized ewes (2.4 ± 0.5 pg/ml) were intermediate. Ovariectomy did not alter concentrations of GnRH receptor mRNA or numbers of GnRH receptors. Treatment of ewes with 1 estradiol implant increased concentrations of GnRH receptor mRNA and numbers of GnRH receptors. In ewes treated with ½ estradiol implant, concentrations of GnRH receptor mRNA were intermediate between controls and ewes treated with 1 estradiol implant, and numbers of GnRH receptors were greater than controls. Luteectomy increased concentrations of GnRH receptor mRNA but did not affect numbers of GnRH receptors. We conclude that estradiol stimulates expression of the GnRH receptor gene and numbers of GnRH receptors in the absence of progesterone. However, effects of estradiol on expression of the GnRH receptor gene were clearly evident only when concentrations of estradiol were elevated to levels typical of the late follicular phase.

Keywords: GnRH receptor; estradiol; sheep

Introduction

Synthesis and secretion of gonadotropic hormones are stimulated following binding of hypothalamic GnRH to specific receptors in the anterior pituitary gland. Therefore, regulation of GnRH receptors represents an important component in the coordination of events that lead to reproductive cyclicity in females. Expression of the GnRH receptor gene and numbers of GnRH receptors are influenced by ovarian steroids. In the ewe, steady-state concentrations of GnRH receptor mRNA (Turzillo et al., 1994; Hamernik et al., 1995) and numbers of GnRH receptors (Moss et al., 1993, 1994; Hamernik et al., 1995) were increased by treatment with estradiol and progesterone. In contrast, progesterone appears to have inhibitory effects on ovine GnRH receptors. In cultured ovine anterior pituitary cells, amounts of GnRH receptor mRNA (Wu et al., 1994) and numbers of GnRH receptors (Laws et al., 1990a) were decreased by treatment with progesterone, and stimulation of concentrations of GnRH receptor mRNA by estradiol were attenuated by progesterone (Sealton et al., 1990). Recently it was reported that treatment of ewes with estradiol does not affect GnRH receptor gene expression during the luteal phase of the estrous cycle, when concentrations of progesterone are high (Brooks & McNeilly, 1994). Potentially opposing effects of estradiol and progesterone are physiologically relevant to the preovulatory period, when changes in circulating levels of these hormones may regulate increases in GnRH receptor gene expression and numbers of GnRH receptors prior to the ovulatory LH surge (Crowder & Nett, 1984; Brooks et al., 1993; Turzillo et al., 1994).

We previously observed increased steady-state concentrations of GnRH receptor mRNA in association with decreased concentrations of circulating progesterone during luteolysis (Turzillo et al., 1994). This change in GnRH receptor gene expression occurred early in the preovulatory period, prior to an increase in circulating concentrations of estradiol. Based on these observations, we postulated that increased expression of the ovine GnRH receptor gene at this time is initiated by removal of inhibitory effects of progesterone and occurs independent of changes in circulating concentrations of estradiol. Subsequently, it was reported by Hamernik et al. (1995) that removal of ovarian steroids for 16 h by ovariectomy failed to affect concentrations of GnRH receptor mRNA in ewes, thus indicating that the continued influence of an ovarian hormone(s) may be required to stimulate GnRH receptor gene expression while concentrations of progesterone are decreasing. Although increased concentrations of circulating estradiol are not requisite for increased GnRH receptor gene expression, it is possible that low levels of estradiol in serum, similar to those observed during the early follicular phase, may have a stimulatory effect on GnRH receptor gene expression following removal of inhibitory effects of progesterone. Therefore, the present study was designed to test the hypothesis that low levels of estradiol in the ewe are sufficient to increase concentrations of GnRH receptor mRNA in the absence of progesterone.

Results

Before treatments were initiated on day 11 or 12 of the estrous cycle, mean serum concentrations of estradiol and progesterone were 1.4 ± 0.2 pg/ml and 2.73 ± 0.2 ng/ml, respectively (n = 24 ewes), and there were no differences among treatment groups (P > 0.55). Circulating concentrations of estradiol and progesterone at the time of pituitary collection are illustrated in Figure 1. Ovariectomy (OVX) did not affect mean concentrations of estradiol in serum. Compared to controls, concentrations of estradiol were elevated (P < 0.05) in ewes that received ½ estradiol implant, and concentrations of estradiol were elevated further in ewes treated with 1 estradiol implant (P < 0.01 compared to ½ estradiol implant). Concentrations of estradiol in luteectomized ewes were intermediate between levels in controls and ewes treated with ½ estradiol implant. Mean circulating concentrations of progesterone decreased following OVX and luteectomy (LUTX) and were >87% lower (P < 0.01) than levels in controls in all other groups at the time of pituitary collection.
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The mean number of LH pulses during the 12 h sampling period was 1.6 ± 0.4 in control ewes. Ovariectomy and LUTX increased (P < 0.001) the frequency of LH pulses to 9.2 ± 1.7 and 8.6 ± 1.0 in 12 h, respectively. Mean numbers of LH pulses were similar between ewes treated with ¼ estradiol implant (5.6 ± 1.6 in 12 h) and ewes treated with 1 estradiol implant (5.3 ± 1.3 in 12 h), and were less (P < 0.01) in estradiol-implanted ewes than in OVX and LUTX ewes.

Twelve h following OVX, mean steady-state concentrations of GnRH receptor mRNA were not different from controls (Figure 2). Concentration of GnRH receptor mRNA were higher (P < 0.01) in ewes that received 1 estradiol implant than in controls. In ewes treated with ¼ estradiol implant, concentrations of GnRH receptor mRNA were intermediate between controls and ewes that received 1 estradiol implant (P = 0.11 compared to controls, Figure 2). LUTX increased (P < 0.05) concentrations of GnRH receptor mRNA compared to controls.

Mean numbers of GnRH receptors were similar between control, OVX and LUTX ewes (Figure 2). Treatment with 1 estradiol implant increased (P < 0.05) numbers of GnRH receptors compared with controls. Numbers of GnRH receptors were higher (P < 0.01) following treatment with 1 estradiol implant than after treatment with ¼ estradiol implant.

Discussion

The endocrine regulation of GnRH receptors most likely involves a precisely coordinated interplay of hypothalamic, ovarian and possibly hypophyseal inputs. The present study focused on the importance of the ovarian steroids estradiol and progesterone in regulating GnRH receptors. Removal of the ovaries resulted in decreased serum concentrations of progesterone but did not affect concentrations of GnRH receptor mRNA or numbers of GnRH receptors. These findings confirm previous results (Hamernik et al., 1995) and indicate that following withdrawal of progesterone, another hormone(s) of ovarian origin may be required to stimulate expression of GnRH receptors.

Replacement of endogenous estradiol with 1 estradiol implant resulted in circulating concentrations of estradiol similar to those observed during the late follicular phase (Karsch et al., 1979; Karsch et al., 1980; Turzillo et al., 1994) and increased concentrations of GnRH receptor mRNA and numbers of GnRH receptors. These results confirm those of previous studies demonstrating the stimulatory effects of estradiol on GnRH receptors in vivo (Moss et al., 1981; Greze & Nett, 1989; Turzillo et al., 1994) and provide further evidence that estradiol is likely important for stimulating maximal expression of GnRH receptors during the later stages of the preovulatory period. However, replacement of circulating levels of estradiol typical of the early follicular phase (¼ estradiol implant) did not increase GnRH receptor gene expression significantly but resulted in concentrations of GnRH receptor mRNA intermediate between controls and ewes with peripheral levels of estradiol typical of the late follicular phase (1 estradiol implant).

Therefore, estradiol may not be the sole factor involved in increasing GnRH receptor gene expression during the early preovulatory period. In LUTX ewes, the endogenous source of progesterone was removed and the endogenous source of estradiol and the remainder of the ovaries was left intact. Despite similar peripheral concentrations of estradiol, a greater and more consistent increase in concentrations of GnRH receptor mRNA was observed in LUTX ewes than in ewes treated with ¼ estradiol implant. Increased concentrations of GnRH receptor mRNA in LUTX ewes were not accompanied by increased numbers of GnRH receptors, and this finding is similar to those of a previous study (Turzillo et al., 1994) in which increased concentrations of GnRH receptor mRNA occurred 12 h following PGF2α-induced luteolysis but increased numbers of GnRH receptors were first noted at 24 h. It is possible that in LUTX ewes, an ovarian hormone other than estradiol contributed to the elevation in concentrations of GnRH receptor mRNA. This idea is supported by previous observations in ovary-intact ewes in which concentrations of GnRH receptor mRNA increased following induction of luteolysis with PGF2α but prior to the increase in circulating concentrations of estradiol (Turzillo et al., 1994). Inhibin has been shown to increase concentrations of GnRH receptor mRNA in cultured ovine pituitary cells (Sealfon et al., 1990), and stimulatory effects of estradiol and inhibin on numbers of GnRH receptors are additive (Gregg et al., 1991; Wu et al., 1994). Since there is evidence that circulating levels of inhibin increase following PGF2α-induced luteolysis (Findlay et al., 1990), it is intriguing to speculate that inhibin of follicular origin together with endogenous estradiol stimulated expression of the GnRH receptor gene in LUTX ewes.

Further experiments are warranted to determine whether inhibin regulates GnRH receptors in vivo.

Despite the lack of significant change in concentrations of GnRH receptor mRNA, numbers of GnRH receptors were increased in ewes treated with ¼ estradiol implant. In previous studies, increased steady-state levels of GnRH receptor mRNA either preceded (Brooks et al., 1993; Turzillo et al., 1994) or accompanied (Brooks & McNelley, 1994; Turzillo et al., 1995b) increases in numbers of GnRH receptors. The discordance between concentration of GnRH recep-

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**Figure 1** Concentrations (mean ± SEM) of estradiol and progesterone in serum collected from control ewes (n = 5); ovariectomized ewes (OVX; n = 5), OVX ewes treated with ¼ estradiol implant (¼ E; n = 5), OVX ewes treated with 1 estradiol implant (1 E; n = 4) and luteectomized ewes (LUTX; n = 5). Significant differences (P < 0.05) among means are indicated by lowercase letters for estradiol and uppercase letters for progesterone.

**Figure 2** Concentrations (mean ± SEM) of GnRH receptor mRNA and GnRH receptors in serum of ovariectomized ewes (OVX; n = 5), OVX ewes treated with ¼ estradiol implant (¼ E; n = 5), OVX ewes treated with 1 estradiol implant (1 E; n = 4) and luteectomized ewes (LUTX; n = 5). Significant differences (P < 0.05) among means are indicated by lowercase letters for concentrations of GnRH receptor mRNA and uppercase letters for concentrations of GnRH receptors.