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Regulation of GnRH receptor gene expression in sheep and cattle

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The GnRH receptor plays a pivotal role in reproduction. This review summarizes current knowledge of the regulation of GnRH receptor gene expression by endocrine factors in sheep and cattle. Expression of the GnRH receptor gene, measured by steady-state amounts of GnRH receptor messenger RNA (mRNA), is maximal during the preovulatory period. The molecular events leading to maximal GnRH receptor gene expression are probably triggered by decreased circulating concentrations of progesterone at luteolysis. Because GnRH is a positive homologous regulator of its own receptor, increased pulsatile GnRH after removal of negative feedback effects of progesterone stimulates expression of the GnRH receptor gene early in the preovulatory period. Oestradiol is also a positive regulator of GnRH receptor gene expression, and increased serum concentrations of oestradiol from developing follicles probably maintain high abundance of GnRH receptor mRNA later in the preovulatory period. Since increased amount of GnRH receptor mRNA precedes maximal numbers of GnRH receptors before the LH surge, increased expression of the GnRH receptor gene is an important mechanism by which maximal sensitivity of gonadotrophs to GnRH is achieved. Future efforts should be directed towards elucidating the molecular mechanisms underlying transcriptional regulation of the GnRH receptor gene in ruminants by endocrine factors.

Introduction

The pituitary receptor for GnRH is a critical component of the reproductive axis. Interaction of GnRH with its receptor stimulates synthesis and secretion of the gonadotrophins that are essential for gonadal function. Numbers of GnRH receptors throughout the oestrous cycle have been characterized (Nett *et al.*, 1987; Nett, 1990), and changes in numbers of GnRH receptors are believed to be important in regulating sensitivity of the anterior pituitary gland to GnRH (Wise *et al.*, 1984). Until 1992, the structural characteristics of the GnRH receptor were unknown and molecular probes for examining regulation of the GnRH receptor gene were not available. Cloning of complementary DNAs (cDNAs) encoding the GnRH receptor in several species including domestic ruminants (Brooks *et al.*, 1993; Kakar *et al.*, 1993) has revealed the basic structure of this receptor. In mice and rats, the GnRH receptor cDNA encodes a 327 amino acid protein while the ovine, bovine, human and pig cDNAs encode 328 amino acids (reviewed by Sealfon *et al.*, 1997). The GnRH receptor is believed to have seven transmembrane domains typical of G protein-coupled receptors. However, the receptor is unique among G protein-coupled receptors in that it lacks a cytoplasmic C-terminal tail (Sealfon *et al.*, 1997). Isolation of these GnRH receptor cDNAs was an essential first step towards increasing understanding of the gene encoding the GnRH receptor, and led to a wide range of studies on the regulation of GnRH receptor gene expression.

This review will focus on the regulation of GnRH receptor gene expression by endocrine factors in ewes. At least four hormones are known to affect the ovine GnRH receptor gene: oestradiol, GnRH, progesterone and inhibin. Evidence for regulation of GnRH receptor gene expression by each of these hormones during the oestrous cycle will be discussed. Although there is much less

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information available regarding GnRH receptor gene expression in the cow, findings from bovine studies are included where appropriate. Current efforts to elucidate molecular mechanisms underlying expression of the GnRH receptor gene in sheep are also discussed.

GnRH Receptor Gene Expression During the Oestrous Cycle

The most dynamic time during the oestrous cycle with respect to pituitary–ovarian interactions is the preovulatory period, when serum concentrations of progesterone decline as a result of luteolysis and serum concentrations of oestradiol and inhibin rise with development of the preovulatory follicle. This pattern of ovarian hormone secretion leads to important changes at the hypothalamus and a marked increase in release of GnRH (Moenter *et al.*, 1991). Throughout the oestrous cycle in ewes and cows, pituitary concentrations of GnRH receptors change four- to tenfold (Nett *et al.*, 1987; Nett, 1990). In ewes, numbers of GnRH receptors remain static during much of the luteal phase, but increase during the preovulatory period (Crowder and Nett, 1984; Brooks *et al.*, 1993; Turzillo *et al.*, 1994; Hamernik *et al.*, 1995). It seems likely that this increase contributes to maximal sensitivity of gonadotrophs to GnRH at this time, and thus is an important step in the series of events leading to the ovulatory LH surge. To determine whether the large number of GnRH receptors during the preovulatory period is the result of increased expression of the GnRH receptor gene, several investigators have measured steady-state concentrations of GnRH receptor mRNA in ovine pituitary glands after induction of luteolysis (Fig. 1). Concentrations of GnRH receptor mRNA are increased as early as 12 h after luteolysis (Turzillo *et al.*, 1994). Amounts of GnRH receptor mRNA remain high at 24 h and 48 h but return to pretreatment (luteal) values at 72 h and 96 h (Brooks *et al.*, 1993; Hamernik *et al.*, 1995). Amounts of GnRH receptor mRNA corresponded closely to numbers of GnRH receptors in each of these studies. In the early preovulatory period, increased GnRH receptor gene expression preceded an increase in the number of GnRH receptors (Fig. 1; Turzillo *et al.*, 1994) and maximal numbers of GnRH receptors were observed later in the preovulatory period near the onset of the LH surge (Crowder and Nett, 1984; Hamernik *et al.*, 1995). These temporal relationships support the hypothesis that increased amounts of ovine GnRH receptor mRNA lead to greater numbers of GnRH receptors which in turn maximize pituitary sensitivity to GnRH in preparation for the LH surge.

An increase in GnRH receptor gene expression has been observed during the preovulatory period in cows (A. M. Turzillo, T. M. Nett, A. Roberts and S. E. Echternkamp, unpublished). However, whether there are concomitant preovulatory increases in numbers of bovine GnRH receptors is unclear, since Leung *et al.* (1984) and Nett *et al.* (1987) did not observe higher concentrations of GnRH receptors during the preovulatory period compared with the luteal phase. However, in both studies, maximal concentrations of GnRH receptors were observed during pro-oestrus. A more detailed study in which pituitary glands are collected at several times after luteolysis is needed to clarify the relationship between GnRH receptor gene expression and numbers of GnRH receptors during the follicular phase in cows.

Collectively, these findings provide strong evidence that the increase in numbers of GnRH receptors during the preovulatory period in sheep and cattle are mediated by increased expression of the GnRH receptor gene. The next logical question is, what are the endocrine factors involved in stimulating GnRH receptor gene expression during the oestrous cycle? In the following sections, how changes in secretion of ovarian and hypothalamic hormones may affect expression of the GnRH receptor gene will be examined.

The Role of Oestradiol

The importance of oestradiol in regulating hypothalamic–pituitary physiology in ruminants is well documented. Treatment with oestradiol can induce an LH surge in ewes and cows, and one mechanism by which oestradiol exerts this effect is by stimulating hypothalamic secretion of GnRH.

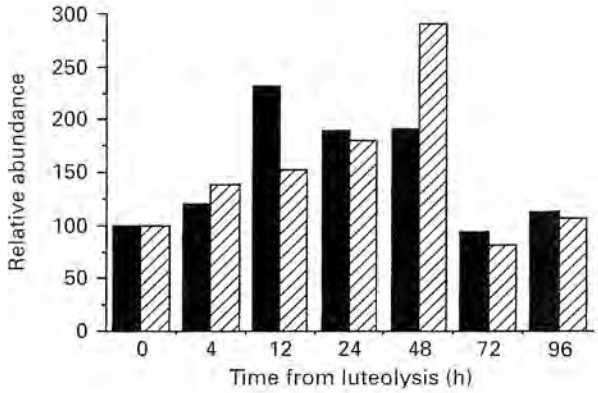

Fig. 1. Relative abundance of GnRH receptor mRNA (mean steady-state levels; ■) and concentrations of GnRH receptors (▨) in anterior pituitary tissue collected from ewes before and after induction of luteolysis. Data were combined from Brooks *et al.* (1993); Turzillo *et al.* (1994); and Hamernik *et al.* (1995).

Oestradiol also has important effects on pituitary function and increases pituitary sensitivity to GnRH in ewes and cows, apparently by increasing numbers of GnRH receptors (Moss *et al.*, 1981; Schoenemann *et al.*, 1985; Turzillo *et al.*, 1994; Hamernik *et al.*, 1995). The stimulatory effect of oestradiol on numbers of GnRH receptors is evident in the absence of hypothalamic input, thus demonstrating a direct pituitary site of action (Gregg and Nett, 1989). Similar effects are observed in cultured ovine pituitary cells, in which oestradiol induces a two- to threefold increase in GnRH-stimulated LH secretion (Huang and Miller, 1980; Moss and Nett, 1980) and increases numbers of GnRH receptors (Laws *et al.*, 1990a; Gregg *et al.*, 1990). More recently, it has been reported that oestradiol increases GnRH receptor gene expression *in vivo* (Turzillo *et al.*, 1994; Hamernik *et al.*, 1995), and this effect can occur in the absence of GnRH (Turzillo *et al.*, 1995b; Adams *et al.*, 1997) and in cultured ovine pituitary cells (Fig. 2; Wu *et al.*, 1994). Because serum concentrations of oestradiol increase markedly during the preovulatory period, these observations appear to indicate that oestradiol is the endocrine factor responsible for increasing GnRH receptor gene expression before the ovulatory LH surge, which in turn leads to greater numbers of GnRH receptors on gonadotrophs.

Although the importance of oestradiol during the preovulatory period is indisputable, there is evidence to indicate that oestradiol may not be the endocrine factor responsible for initiating the events leading to maximal numbers of GnRH receptors. To characterize the temporal relationships among GnRH receptor gene expression and endocrine changes during the early preovulatory period, we measured serum concentrations of oestradiol and progesterone and amounts of GnRH receptor mRNA in ewes during the 24 h after induction of luteolysis with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Turzillo *et al.*, 1994). Concentrations of GnRH receptor mRNA were increased at 12 h after treatment with $PGF_{2\alpha}$. This increase was associated with a 50% decrease in circulating concentrations of progesterone, but occurred before serum concentrations of oestradiol began to rise (Fig. 3). Thus it appears that the initial molecular events leading to increased expression of the GnRH receptor gene during the early preovulatory period in ewes do not require increased pituitary exposure to oestradiol, but instead are associated more closely with decreased concentrations of progesterone. Oestradiol probably maintains increased GnRH receptor gene expression later in the preovulatory period. Therefore, progesterone may exert negative effects on GnRH receptor gene expression during the oestrous cycle, which led us to consider the potential mechanisms underlying this effect of progesterone.

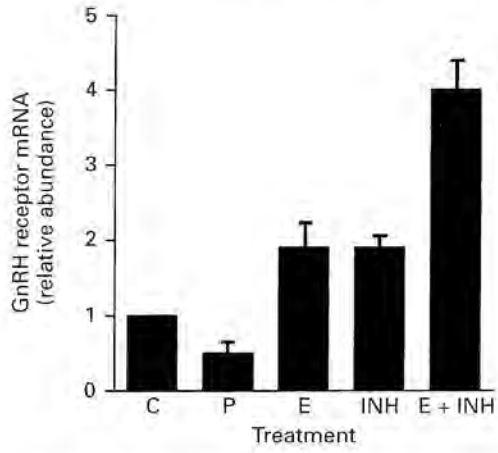

Fig. 2. Mean (± SEM) concentrations of GnRH receptor mRNA in cultured ovine pituitary cells treated for 48 h with control medium (C), progesterone (P; 100 nmol l^{-1}), oestradiol (E; 10 nmol l^{-1}), an enriched preparation of porcine follicular inhibin (INH; equivalent to 10 ng ml^{-1} pure 32 kDa porcine inhibin), or the combination of E + INH. Adapted from Wu *et al.* (1994).

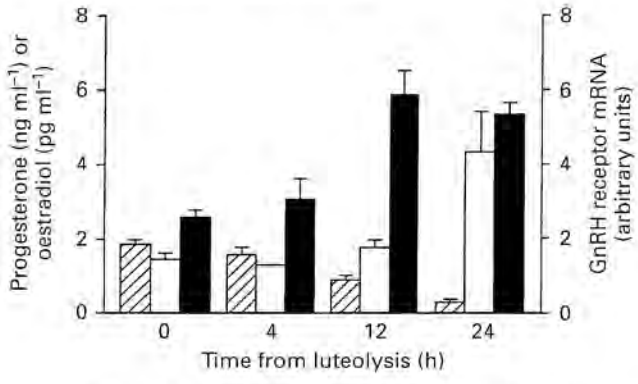

Fig. 3. Concentrations of progesterone (▨) and oestradiol (□) in serum and GnRH receptor mRNA in pituitary tissue (■) collected from ewes 0 h, 4 h, 12 h and 24 h after induction of luteolysis with $PGF_{2\alpha}$. Data are means ± SEM. Note that increased concentrations of GnRH receptor mRNA at 12 h occurred before serum concentrations of oestradiol were increased and were associated with a 50% decrease in serum concentrations of progesterone. Adapted from Turzillo *et al.* (1994).

Progesterone: A Negative Regulator of GnRH Receptor Gene Expression

Several lines of evidence implicate progesterone as a negative regulator of ovine GnRH receptor gene expression. First, numbers of GnRH receptors and expression of the GnRH receptor gene are relatively low during the luteal phase when concentrations of progesterone are maximal, and

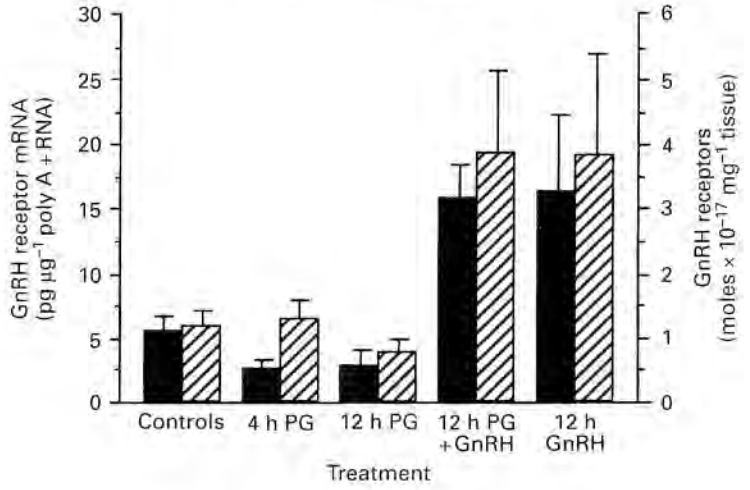

Fig. 4. Pituitary concentrations of GnRH receptor mRNA (■) and GnRH receptors (▨) in ewes during the anoestrous season. Follicular growth and ovulation were stimulated pharmacologically, and luteolysis was induced 11 or 12 days later with $PGF_{2\alpha}$. Pituitary tissues were collected 4 h or 12 h after treatment with $PGF_{2\alpha}$ (PG), 12 h after treatment with $PGF_{2\alpha}$ and GnRH (12 h PG + GnRH), or 12 h after treatment with GnRH only (12 h GnRH). GnRH was administered at intervals of 1 h. Control ewes were not treated with $PGF_{2\alpha}$ or GnRH. Data are means ± SEM. Adapted from Turzillo *et al.* (1995b).

increase when progesterone falls during the demise of the corpus luteum. Second, the stimulatory effects of oestradiol on GnRH receptor gene expression and numbers of GnRH receptors are prevented during the luteal phase when endogenous progesterone is high (Brooks and McNeilly, 1994; Turzillo *et al.*, 1998). The third line of evidence comes from a series of studies conducted *in vitro*. In cultured ovine pituitary cells, progesterone decreases responsiveness to GnRH (Batra and Miller, 1985), numbers of receptors for GnRH (Laws *et al.*, 1990b) and amounts of mRNA encoding the GnRH receptor (Fig. 2; Wu *et al.*, 1994). Furthermore, progesterone can attenuate the stimulatory effects of oestradiol on GnRH-stimulated LH secretion (Batra and Miller, 1985) and numbers of GnRH receptors (Sealfon *et al.*, 1990) *in vitro*. From these studies it seems logical to hypothesize that progesterone has inhibitory effects on GnRH receptors *in vivo*, and that the decrease in circulating concentrations of progesterone at luteolysis removes this inhibition, allowing increased expression of the GnRH receptor gene followed by increased numbers of GnRH receptors.

There are at least two mechanisms by which progesterone might exert its effects on the GnRH receptor gene. One mechanism involves direct effects of progesterone on the pituitary gland. This mechanism seems plausible in the light of evidence that progesterone affects GnRH receptor gene expression *in vitro*, in the absence of hypothalamic influences. Alternatively, progesterone might regulate the GnRH receptor gene indirectly via negative feedback effects on secretion of GnRH. Decreasing concentrations of progesterone during luteolysis lead to increased pulsatile secretion of GnRH. As discussed later, GnRH is an important regulator of its own receptor and pulsatile GnRH increases the number of GnRH receptors in ewes (Khalid *et al.*, 1987; Hamernik and Nett, 1988; Turzillo *et al.*, 1995b). Therefore, increased stimulation of the pituitary gland by GnRH during luteolysis could lead to increased expression of the GnRH receptor gene.

To determine whether progesterone affects GnRH receptor gene expression *in vivo* directly at the pituitary or indirectly via increased GnRH secretion, we designed an experiment in which decreased concentrations of progesterone could be achieved without a concurrent rise in pulsatile GnRH (Turzillo *et al.*, 1995b). Ovulation and subsequent luteal formation were induced in anoestrous ewes and luteolysis was initiated on day 11 or 12 of the induced oestrous cycle. Because of photoperiodic

inhibition of the GnRH pulse generator during anoestrus, an increase in GnRH secretion following luteolysis did not occur in this experimental model. If effects of progesterone on GnRH receptor gene expression occur directly at the pituitary gland and are independent of GnRH, decreasing concentrations of progesterone in this model should result in increased amounts of GnRH receptor mRNA. This was not the case (Fig. 4). In contrast, when pulsatile GnRH was administered each hour for 12 h after induction of luteolysis, concentrations of GnRH receptor mRNA were increased. This effect of GnRH was also obvious in ewes in which luteolysis was not induced and serum concentrations of progesterone remained high (Fig. 4). From these results, we conclude that the increase in GnRH receptor mRNA during the early preovulatory period is mediated by an indirect mechanism involving decreased negative feedback of progesterone on pulsatile hypothalamic GnRH secretion. Increased stimulation of the pituitary gland by GnRH then causes heightened expression of the GnRH receptor gene, thus initiating the molecular events that lead to increased concentrations of GnRH receptor mRNA and synthesis of GnRH receptors before the LH surge.

Homologous Regulation by GnRH

GnRH is secreted from the ovine hypothalamus in a pulsatile fashion and the importance of this pattern of GnRH secretion to pituitary function is well established. A marked increase in GnRH release occurs during the follicular phase in ewes (Moenter *et al.*, 1991), and this increase is required for a normal LH surge. In addition to stimulating pulsatile LH secretion during the preovulatory period, GnRH is also a homologous regulator of its own receptor and probably serves to increase numbers of GnRH receptors before the ovulatory LH surge. Sheep have been used to develop several excellent experimental paradigms to study the regulation of GnRH receptors by GnRH. One of these paradigms takes advantage of the large size of sheep, which allows surgical disconnection of the hypothalamus from the pituitary gland (hypothalamic–pituitary disconnection, HPD). This procedure effectively deprives the pituitary gland of GnRH (and other hypothalamic hormones), but does not interfere with the hypophyseal blood supply and allows the pituitary gland to remain viable despite the absence of hypothalamic input. In ovariectomized ewes, HPD results in decreased numbers of GnRH receptors (Clarke *et al.*, 1987; Gregg and Nett, 1989; Turzillo *et al.*, 1995a) which can be restored by treatment with pulsatile GnRH (Clarke *et al.*, 1987; Hamernik and Nett, 1988). Significant reductions in numbers of GnRH receptors also occur when the pituitary gland is deprived of GnRH using non-surgical methods. Brooks and McNeilly (1994) observed decreased numbers of GnRH receptors after treating ewes during the oestrous cycle with an antagonist of GnRH, and Sakurai *et al.* (1997) reported similar results in wethers passively immunized against GnRH. From these studies, it is clear that continuous stimulation of the pituitary gland by GnRH is required to maintain normal numbers of GnRH receptors. However, there is some discrepancy regarding the effect of removing GnRH on GnRH receptor gene expression. Brooks and McNeilly (1994) and Sakurai *et al.* (1997) found that treatment of ovary-intact ewes with GnRH antagonist or passive immunization of wethers against GnRH resulted in decreased concentrations of GnRH receptor mRNA. This is in contrast to our observations in ovariectomized ewes in which amounts of GnRH receptor mRNA did not change significantly during the 3 days after HPD (Turzillo *et al.*, 1995a) or 6 days after passive immunization against GnRH (Turzillo and Nett, 1997). Although reasons for the lack of agreement among these studies are not obvious, the disparate results may be due to the use of different experimental models and the time after removal of GnRH when measurements were made.

Despite different effects of removing GnRH on GnRH receptor gene expression, administration of pulsatile GnRH to GnRH-deficient anoestrous ewes increased pituitary amounts of GnRH receptor mRNA (Turzillo *et al.*, 1995b) and numbers of GnRH receptors (Khalid *et al.*, 1987; Hamernik and Nett, 1988; Turzillo *et al.*, 1995b). These observations strengthen the claim that pulsatile GnRH is a positive regulator of GnRH receptor gene expression.

Information regarding effects of pulsatile GnRH on bovine GnRH receptors is limited. Numbers of GnRH receptors were increased by pulsatile GnRH in prepubertal bull calves (Rodriguez and

Wise, 1991) but in nutritionally anoestrous cows, treatment with pulsatile GnRH did not affect pituitary concentrations of GnRH receptors or GnRH receptor mRNA (Vizcarra *et al.*, 1997). Because secretion of LH is reduced in nutritionally anoestrous cows (Richards *et al.*, 1989), it is presumed that release of GnRH is also reduced. However, it is possible that the decrease in GnRH caused by nutritional restriction in the study of Vizcarra *et al.* (1997) was sufficient to affect LH secretion but not GnRH receptors. This result could explain why treatment with exogenous GnRH pulses was ineffective in stimulating further increases in pituitary concentrations of GnRH receptors or GnRH receptor mRNA.

Collectively, the bulk of evidence indicates that pulsatile stimulation by GnRH is required to maintain tissue concentrations of GnRH receptor and GnRH receptor mRNA in the ruminant pituitary gland. Therefore, marked increases in GnRH secretion probably play an important role in upregulation of GnRH receptors during the preovulatory period.

It is important to note that effects of GnRH on GnRH receptors differ markedly depending on the pattern of GnRH administration. After continuous exposure to GnRH, the anterior pituitary gland becomes refractory to further challenge with GnRH in ewes (Nett *et al.*, 1981) and cows (Lamming and McLeod, 1988). This desensitization is marked by decreased tissue concentrations of GnRH receptors (Nett *et al.*, 1981; Crowder *et al.*, 1986; Vizcarra *et al.*, 1997) and GnRH receptor mRNA (Vizcarra *et al.*, 1997; Turzillo *et al.*, 1998). Similarly, chronic treatment of ewes or wethers with GnRH agonists causes downregulation of GnRH receptors and reduces GnRH receptor gene expression (Brooks and McNeilly, 1994; Wu *et al.*, 1994). GnRH receptors are internalized after binding GnRH (Hazum *et al.*, 1980), and it is likely that the reduction in numbers of GnRH receptors induced by continuous GnRH treatment reflects this internalization. However, recent observations that decreased GnRH receptor gene expression occurs in conjunction with downregulation of GnRH receptors provide evidence that pituitary desensitization following continuous exposure to GnRH is mediated by reduced *de novo* synthesis of GnRH receptors as well as by internalization of existing GnRH receptors on gonadotrophs. These findings may have important physiological relevance. Secretion of GnRH increases 40-fold during the preovulatory period in ewes, and remains high for several hours after the LH surge (Moenter *et al.*, 1991). Since concentrations of GnRH receptor mRNA and numbers of GnRH receptors decrease after the LH surge (Crowder and Nett, 1984; Hamernik *et al.*, 1995), we speculate that termination of the LH surge may be due to pituitary desensitization mediated by decreased GnRH receptor gene expression and downregulation of GnRH receptors caused by extended exposure to high concentrations of GnRH.

Does Inhibin Regulate GnRH Receptor Gene Expression?

Inhibin is a gonadal peptide that selectively suppresses secretion of FSH. Inhibin is secreted by large antral ovine and bovine follicles, and increases in circulating concentrations of inhibin during the follicular phase in sheep and cattle have been reported (Padmanabhan *et al.*, 1984; Findlay *et al.*, 1990). Therefore, like oestradiol, ovarian inhibin may be an important endocrine regulator of pituitary function during the preovulatory period. Evidence that inhibin may affect GnRH receptors comes from studies conducted *in vitro*. In cultured ovine pituitary cells, inhibin enhanced GnRH-stimulated secretion of LH (Miller and Huang, 1985; Muttukrishna and Knight, 1990), increased numbers of GnRH receptors (Laws *et al.*, 1990b; Gregg *et al.*, 1991) and increased amounts of GnRH receptor mRNA (Fig. 2; Wu *et al.*, 1994). In the study by Wu *et al.* (1994), the combination of oestradiol and inhibin resulted in an additive effect on amounts of GnRH receptor mRNA. Positive effects of inhibin *in vitro* support the hypothesis that increasing concentrations of inhibin during the preovulatory period contribute to increased GnRH receptor gene expression and greater numbers of GnRH receptors observed during this time. However, there is no evidence to suggest that inhibin regulates GnRH receptors *in vivo*. In fact, treatment of ewes during the luteal phase for 4 or 9 days with bovine or ovine follicular fluid as a source of inhibin did not affect tissue concentrations of GnRH receptors or GnRH receptor mRNA (Brooks *et al.*, 1992; Brooks and McNeilly, 1994). Similarly, we observed no changes in numbers of GnRH receptors or GnRH receptor gene expression in

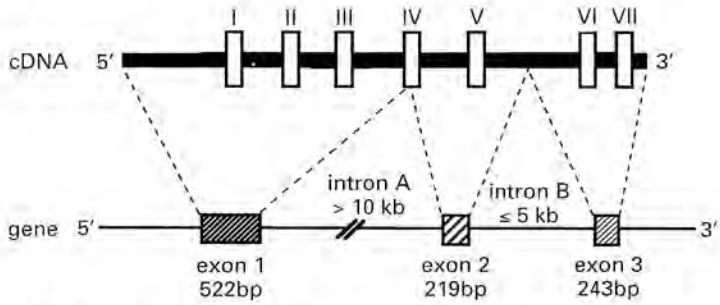

Fig. 5. Illustration of complementary DNA (cDNA) and gene encoding the ovine GnRH receptor. Transmembrane domains of the cDNA are represented by roman numerals I–VII. The coding sequence of the receptor is divided among three exons and two introns. Sizes of exons and introns are indicated in base pairs (bp) or kilobases (kb), respectively. (Reproduced with permission from Campion *et al.*, 1996).

ovariectomized ewes treated with bovine follicular fluid (Turzillo and Nett, 1997). The absence of an effect of treatment with bovine follicular fluid cannot be explained by a lack of inhibin bioactivity since serum concentrations of FSH and pituitary amounts of mRNA encoding FSHβ subunit were reduced ≥ 70% and 90%, respectively. Collectively, the results of studies conducted *in vivo* do not support the idea that inhibin is an endocrine regulator of GnRH receptors. Reasons for the lack of agreement between findings obtained *in vitro* versus *in vivo* are unclear, but may be related to the potential role of inhibin as an intra-pituitary regulatory factor. Inhibin subunits are produced in rat gonadotrophs (Roberts *et al.*, 1988), and local production of these subunits may be involved in transcriptional activation of the GnRH receptor gene (Fernandez-Vazquez *et al.*, 1996). Whether there is a functional system involving paracrine or autocrine actions or inhibin in the ruminant pituitary gland is not yet certain. However, it is possible that the effects of inhibin on numbers of GnRH receptors and GnRH receptor gene expression *in vitro* may reflect intra-pituitary regulation rather than endocrine effects of inhibin of ovarian origin.

The Gene Encoding Ovine GnRH Receptor

From the preceding paragraphs, it is easy to understand how isolation of cDNAs encoding GnRH receptors has expanded knowledge of GnRH receptor gene expression in cattle and sheep. Concurrent with studies on the endocrine regulation of GnRH receptor gene expression, we became interested in exploring the molecular mechanisms underlying transcriptional regulation of the GnRH receptor gene in ruminants. To begin to address this issue, we cloned the ovine GnRH receptor gene (Fig. 5; Campion *et al.*, 1996). This gene comprises three exons and two introns, and occurs as a single copy gene. Although there is considerable identity (≥ 60%) between the nucleotide sequences of the proximal 5′ flanking regions of the ovine and murine (Zhou *et al.*, 1994; Clay *et al.*, 1995) GnRH receptor genes, there are striking differences among species with respect to the DNA regulatory elements required for cell-specific expression. Transcriptional activity of the murine GnRH receptor gene is conferred by three cis-acting elements that lie within 500 bp of the proximal promoter (Duval *et al.*, 1997). These elements include an activating protein-1 (AP-1) binding site, presumably activated by the fos/jun family of transcription factors; a binding site for the orphan nuclear receptor, steroidogenic factor 1 (SF-1; reviewed by Parker and Schimmer, 1997); and GnRH receptor activating sequence, for which the trans-acting factor has yet to be identified. Of these three elements, only SF-1 appears to be a conserved mechanism for regulation of the ovine GnRH receptor promoter. Transcriptional activity of this promoter is increased following transient co-transfection of COS-7 cells with an expression vector for SF-1 (Quirk, 1997), indicating that SF-1 may regulate

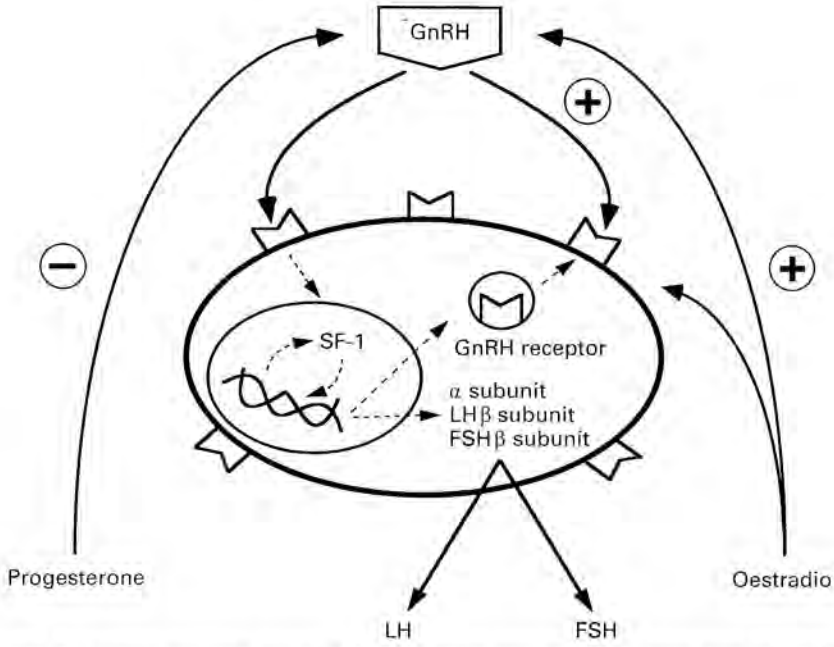

Fig. 6. Current working model of the endocrine and molecular regulation of GnRH receptor gene expression in gonadotrophs of domestic ruminants. Pulsatile GnRH from the hypothalamus stimulates GnRH receptor gene expression, and this effect appears to be modulated by negative feedback effects of progesterone from the corpus luteum. Oestradiol from the ovary also stimulates expression of the GnRH receptor gene. Although this effect of oestradiol is exerted directly on the pituitary gland, increased secretion of GnRH during positive feedback by oestradiol may also contribute to increased GnRH receptor gene expression. The transcription factor steroidogenic factor 1 (SF-1) may be an important regulator of several genes expressed by ruminant gonadotrophs, including the GnRH receptor gene. Increased expression of the GnRH receptor gene is believed to result in synthesis of GnRH receptors and their expression on the plasma membrane of gonadotrophs. Since interaction of GnRH with its receptor is absolutely necessary for synthesis of the gonadotrophin subunits (α, LHβ and FSHβ, regulated expression of the GnRH receptor gene and synthesis of GnRH receptors are critical steps in the production of LH and FSH.

transcription of the GnRH receptor gene in sheep. In the light of evidence that SF-1 also regulates transcription of the bovine LHβ subunit gene (Keri and Nilson., 1996) and the gene for SF-1 is expressed in ovine gonadotrophs (Turzillo *et al.*, 1997), SF-1 is emerging as a possible common transcriptional regulator of several genes that define the ruminant gonadotrope.

In addition to cell-specific expression, it is of interest to identify potential molecular mechanisms that mediate responsiveness of the GnRH receptor gene to endocrine factors. A DNA element that confers responsiveness of the ovine GnRH receptor gene to changes in concentrations of intracellular cAMP has been identified (C. M. Clay, personal communication). Transcriptional activity of the proximal promoter is increased in the presence of forskolin (a pharmacological activator of adenylyl cyclase), and this response appears to be mediated via a cAMP response element capable of binding cAMP response element binding protein. These findings implicate cAMP as a potential regulator of the GnRH receptor gene. In cultured ovine pituitary cells, binding of GnRH to ovine gonadotrophs increases cAMP and addition of a cAMP derivative can mimic the LH-releasing effect of GnRH (Adams *et al.*, 1979). Furthermore, treatment of rat pituitary cells with analogues of cAMP increased numbers of GnRH receptors (Young *et al.*, 1984). Thus it is intriguing to speculate that the stimulatory effects of GnRH on tissue concentrations of GnRH receptor mRNA and GnRH receptors

in sheep and cattle may be mediated at the molecular level by cAMP. Additional studies are needed to explore further the role of cAMP in regulation of GnRH receptor gene expression by GnRH and other endocrine factors.

Conclusion

Coordinated changes in sensitivity of the anterior pituitary gland to GnRH are required for normal reproductive cyclicity in cattle and sheep. Therefore, knowledge of the mechanisms regulating GnRH receptors is not only valuable to our basic understanding of pituitary function, but is also relevant to the development of improved methods for controlling fertility in domestic ruminants. We and others have characterized patterns of GnRH receptor gene expression and the endocrine regulation of these patterns during the oestrous cycle in sheep and cattle. It is clear that both hypothalamic (GnRH) and ovarian hormones (progesterone and oestradiol) influence the expression of the GnRH receptor gene and numbers of GnRH receptors (Fig. 6). Because of the discrepancy regarding effects of inhibin on GnRH receptor gene expression *in vitro* versus *in vivo*, the role of ovarian inhibin as an endocrine regulator of the GnRH receptor gene in domestic ruminants remains uncertain. Recent evidence indicates that SF-1 may be a common transcriptional regulator of several genes expressed by gonadotrophs in domestic ruminants. Isolation of the gene encoding the ovine GnRH receptor will allow further study of the molecular mechanisms by which transcription of the GnRH receptor gene is influenced by changes in the endocrine milieu.

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