ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 1996, 56: 727-741

Neural systems mediating the negative feedback actions of estradiol and progesterone in the ewe

Robert L. Goodman

Department of Physiology, West Virginia University Health Sciences Center, P.O. Box 9229, Morgantown, WV 26506-9229, USA

Abstract. The ewe shows a marked seasonal variation in the effects of ovarian steroids on pulsatile GnRH secretion. In the breeding season, progesterone inhibits GnRH pulse frequency, while estradiol suppresses pulse amplitude. In anestrus, both steroids inhibit pulse frequency. The effects of progesterone in both seasons are mediated by endogenous opioid peptides (EOP) that act in the preoptic area (POA) and medial basal hypothalamus (MBH). However, knife cut studies indicate that actions in the MBH are most important. Moreover, blockade of EOP receptors activates (e.g., induces Fos) GnRH perikarya in the MBH, but not those in the POA. Thus interactions between EOP and GnRH neurons within the MBH may be critical for progesterone negative feedback. The neural systems mediating estradiol suppression of GnRH pulse amplitude in the breeding season are largely unknown, although α-adrenergic neurons may be involved. The seasonal variation in inhibition of GnRH pulse frequency by estradiol is postulated to be mediated by a group of dopaminergic (DA) neurons that have three important properties: (1) they inhibit GnRH pulse frequency; (2) their activity is stimulated by estradiol; and (3) they are functional in anestrus, but not the breeding season. Recent work examining the effects of lesions of DA neurons and the ability of estradiol to induce Fos in DA cells strongly suggests that DA neurons in the retrochiasmatic area (A15) and POA (A14) have all three characteristics. We thus propose that these DA neurons are responsible for the seasonal variation in the ability of estradiol to inhibit GnRH pulse frequency.

Mini-review

Key words: estradiol, progesterone, LH pulses, GnRH pulses, negative feedback, seasonal breeding, ewe, endogenous opioids, dopamine, norepinephrine

INTRODUCTION

The existence of a negative feedback loop between pituitary gonadotropins and ovarian hormones was first proposed in 1932 (Moore and Price). In the intervening 60 years, reproductive endocrinologists have: (1) identified the two pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and described in detail the effects of each of these on the ovary; (2) isolated and determined the structures of the primary ovarian steroids, estradiol (E_2) and progesterone and determined their mechanism of action; (3) recognized the difference between tonic and surge secretion of LH, that the former controls steroidogenesis while the latter induces ovulation, and that ovarian steroids have markedly different effects on these two modes of secretion (e.g., E_2 inhibits tonic LH secretion, but induces the LH surge); (4) realized that the brain controls anterior pituitary function, identified gonadotropin-releasing hormone (GnRH), determined the distribution and innervation of GnRH neurons, and recognized the importance of episodic GnRH release; (5) described the control mechanisms responsible for the ovarian cycle in a number of species; and (6) described the changes in control of gonadotropins and GnRH that are responsible for the suppression of ovarian function prior to puberty and annually in seasonal breeders. Despite this impressive list of accomplishments, we still know very little about some aspects of the negative feedback relationship between the ovarian steroids and LH. As noted above, we know that this feedback loop involves tonic LH secretion and understand the mechanisms by which tonic LH secretion controls ovarian production of E_2 and progesterone. On the other hand, the mechanisms by which ovarian steroids feedback inhibit tonic LH secretion remain largely unknown. For example, whether gonadal steroids produce any inhibitory effects on tonic GnRH secretion in the rat (Kalra and Kalra 1989) or the monkey (Nakai et al. 1978) remains controversial. In contrast, in the ewe, direct measurement of GnRH in hypophyseal portal blood has unequivocally demonstrated that both E_2 and progesterone inhibit tonic GnRH secretion (Clarke et al. 1987, Karsch et al. 1987, Moenter et al. 1991, Evans et al. 1994, Goodman et al. 1995b). GnRH neurons, however, contain few, if any, steroid receptors in the ewe (Herbison et al. 1993, Lehman and Karsch 1993) or other species (Silverman et al. 1994). Thus, any inhibitory actions of E_2 and progesterone on GnRH secretion must be mediated by other neural systems. In this article, I will review our current knowledge of the neural systems mediating the negative feedback actions of ovarian steroids in the ewe. Because of space considerations and earlier reviews (Martin 1984, Karsch 1987, Goodman 1994), this article will focus on more recent work addressing this issue.

INTERACTION OF SEASON AND STEROIDS IN THE CONTROL OF PULSATILE GnRH SECRETION

Two important characteristics of the hypothalamo-hypophyseal-ovarian axis in the ewe need to be kept in mind when considering the negative feedback actions of ovarian steroids. First, reproductive function in the ewe shows a seasonal variation. In most breeds of sheep, ewes will show regular 16-17 day estrous cycles in the fall and winter, but will be anovulatory (or anestrous) during the spring and summer. As reviewed in detail elsewhere (Karsch et al. 1984, Goodman 1994), this annual cycle in reproductive function is controlled by the external photoperiod, acting *via* melatonin secretion from the pineal gland. The pattern of melatonin secretion, in turn, controls ovarian function by producing a seasonal variation in the response to E_2 negative feedback: during the breeding season, physiological E_2 levels produce only a modest suppression of tonic LH secretion, while in anestrus the same E_2 levels markedly inhibit LH release (Legan et al. 1977). Thus, any discussion of the mechanisms mediating E_2 negative feedback must consider this photoperiod--controlled alteration in the inhibitory effects of E_2.

The other important characteristic of the hypothalamo-hypophyseal-ovarian axis is that GnRH is

usually released from the hypothalamus in an episodic pattern (Moenter et al. 1992b), with each pulse of GnRH producing a corresponding brief episode of LH release from the anterior pituitary. As a result, peripheral LH concentrations show a pulsatile pattern (see Fig. 1), with each LH pulse characterized by an abrupt, brief increase in LH concentrations followed by a prolonged exponential decay as LH is removed from the circulation. It should be noted that during the LH surge, GnRH secretion occurs continuously, at least in the ewe (Moenter et al. 1992b). Nevertheless, it is clear based on LH pulse patterns that tonic GnRH and LH secretion occurs episodically in a wide variety of species and under virtually all endocrine conditions.

Although tonic GnRH and LH secretion is always episodic, the characteristics of the pulse pattern varies, depending on the endocrine condition of the animal. This variability largely reflects the negative feedback actions of ovarian steroids, which changes with stage of the estrous cycle and with season. This interaction between season and steroids to control LH pulse patterns is illustrated in Fig. 1. During the breeding season, E_2 and progesterone both decrease tonic LH secretion relative to untreated ovariectomized (OVX) ewes, but they do so by different mechanisms: progesterone inhibits LH pulse frequency, while E_2 inhibits LH pulse amplitude (Goodman and Karsch 1980). E_2 also produces a slight increase in LH pulse frequency, but the physiological significance of this effect remains unclear (Karsch et al.1983). In contrast, during the anestrous season (Fig. 1) both E_2 and progesterone inhibit LH pulse frequency (Goodman et al. 1982, Martin et al. 1983). It is important to note that this seasonal variation in the effects of E_2 on LH pulse frequency correlates with the variation in E_2 negative feedback described above. During the breeding season, when E_2 is a weak inhibitory steroid, it inhibits LH pulse amplitude but cannot inhibit pulse frequency; during anestrus, when E_2 produces a potent suppression of tonic LH secretion it does so by inhibiting LH pulse frequency. Moreover, it is now clear that this seasonal variation in the effects of E_2 on LH pulse frequency is controlled by photoperiod (Robinson et al. 1985) and can account for the seasonal alterations in ovulation in ovary-intact ewes (Goodman and Karsch 1981).

In light of these differences in the negative feedback actions of ovarian steroids, it is useful to consider separately the neural systems mediating each of them. Thus, in the remainder of this review, I will consider the neural systems involved in: (1) the inhibition of LH pulse frequency by progesterone; (2) the inhibition of LH pulse amplitude by E_2 in breeding season ewes; and (3) the inhibition of LH pulse frequency by E_2 in anestrous ewes.

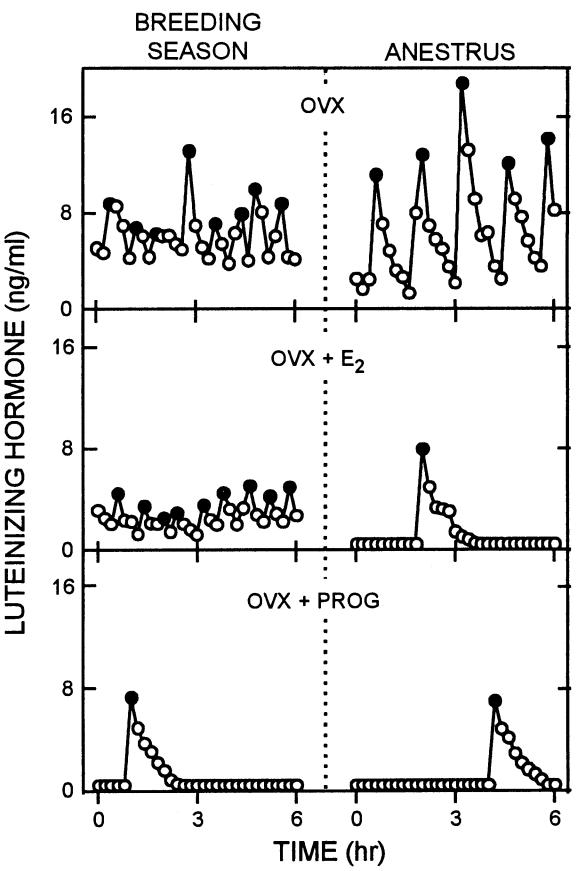

Fig. 1. LH pulse patterns from representative OVX (top panels), E_2-treated OVX (middle panels), and progesterone-treated OVX (bottom panels) ewes during the breeding (left panels) and anestrous (right panels) seasons. Solid circles depict peaks of LH pulses. Data redrawn from Goodman and Karsch 1980 and Goodman et al. 1982.

NEURAL SYSTEMS MEDIATING PROGESTERONE NEGATIVE FEEDBACK

There is now general agreement that the negative feedback action of progesterone is mediated, at least in part, by endogenous opioid peptides (EOP). This consensus is based on numerous reports that EOP agonists, including β-endorphin, inhibit pulsatile LH secretion when progesterone is absent (Brooks et al. 1986b, Curlewis et al. 1991b) and that EOP antagonists increase LH pulse frequency in luteal-phase ewes and in progesterone-treated OVX (OVX+P) ewes (Brooks et al. 1986b, Trout and Malven 1987, Whisnant and Goodman 1988, Yang et al. 1988). Since these antagonists have little, if any, effect on LH pulse frequency in the absence of progesterone (Trout and Malven 1987,Whisnant and Goodman 1988, Yang et al. 1988), progesterone appears to be acting by increasing EOP tone, which then inhibits LH pulse frequency. Further, since the effects observed were on LH pulse frequency, it was inferred that progesterone and EOP antagonists were altering GnRH pulse frequency, a conclusion that has since been confirmed by direct measurements of GnRH (Clarke et al. 1987, Horton et al. 1987, Karsch et al. 1987, Moenter et al. 1991). It is also interesting to note that these EOP systems are present in anestrous ewes, although (in the absence of corpora lutea at this time of year) they are only active if exogenous progesterone is provided (Brooks et al. 1986a).

While it is clear the EOP systems play an important role in progesterone negative feedback, the specific EOP neurons involved remain to be identified. The effects of local administration of EOP antagonists to different hypothalamic areas suggest that EOP can act in both the preoptic area (POA) and medial basal hypothalamus (MBH) to inhibit LH pulse frequency in luteal-phase ewes (Malven et al. 1990, Whisnant et al. 1991, Conover et al. 1993). A similar study using antisera to EOP led to the conclusion that β-endorphin acts in the POA, while met-enkephalin acts in the MBH (Weesner and Malven 1990); but it is also clear that β-endorphin can act in the median eminence to inhibit GnRH pulse frequency (Conover et al. 1993). Finally, progesterone treatment of OVX ewes increases mRNA levels for the precursor to β-endorphin in the MBH, so that this steroid may stimulate β-endorphin perikarya in the region of the arcuate (Whisnant et al. 1993).

While these studies suggest that EOP can act in both the POA and MBH to mediate the negative feedback action of progesterone, we have recently obtained two lines of evidence that the MBH is more important. In the first experiment, we examined the effects of anterior deafferentation between the POA and MBH on the negative feedback actions of progesterone (Whisnant and Goodman 1994). These knife cuts had no effect on either the ability of progesterone to inhibit LH pulse frequency in OVX ewes or the ability of an EOP antagonist to increase LH pulse frequency in OVX+P ewes (Fig. 2). In contrast, these cuts completely blocked the negative feedback action of E_2 during anestrus (Whisnant and Goodman 1994). It thus appears that connections between the POA and MBH are not essential for progesterone to inhibit GnRH pulse frequency *via* EOP. This agrees with previous studies in which similar knife cuts had no obvious effect on the feedback loop controlling progesterone secretion in luteal phase ewes (Pau et al. 1982). In the second experiment, we attempted to identify the GnRH neurons inhibited by EOP during the luteal phase. This was done by monitoring expression of the early immediate gene product, Fos, in GnRH perikarya after injection of an antagonist to block EOP inhibition of GnRH. Fos has been used extensively as an index of neuronal activation (Dragunow and Faull 1989) because it can be co-localized with specific neurotransmitters using a dual immunocytochemical procedure (Lehman et al. 1996). In this study, Fos and GnRH were examined in tissues collected two hours after iv injection of an EOP antagonist to luteal phase ewes (Goodman et al. 1995a). As expected this antagonist increased LH pulse frequency. There was a corresponding increase in Fos expression in MBH GnRH neurons; 32 ± 15% of GnRH neurons in the MBH contained Fos in the antagonist-treated ewes, compared to 8 ± 6% in con-

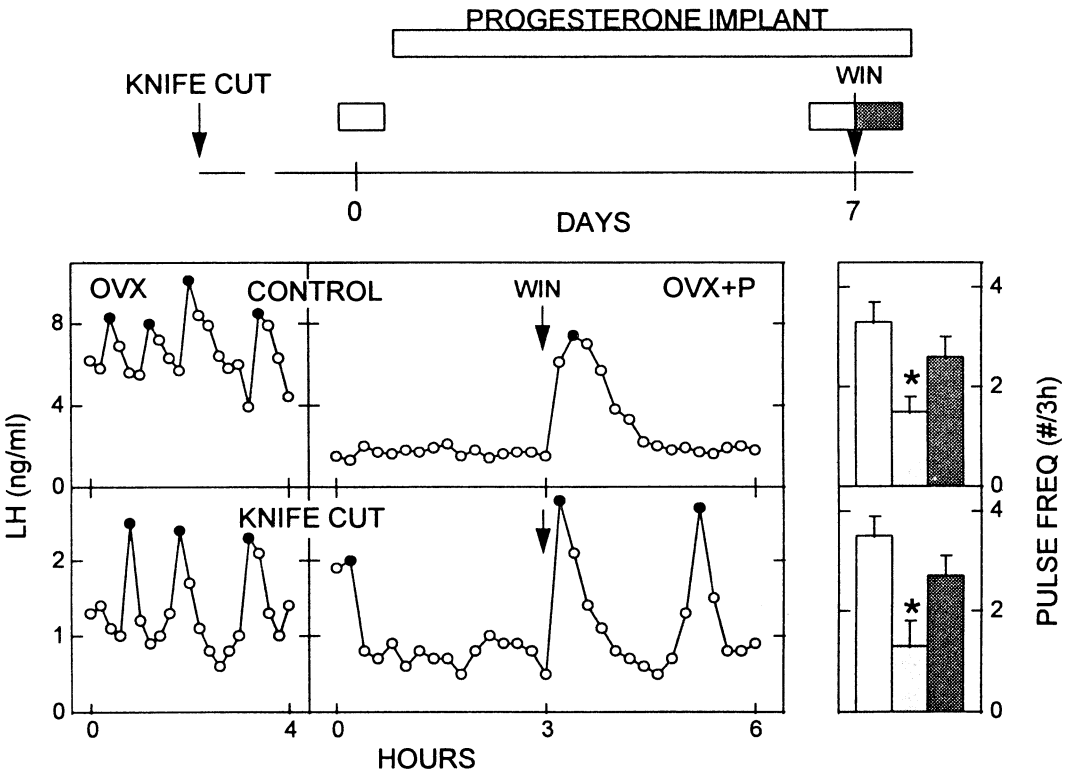

Fig. 2. Effect of anterior deafferentation on progesterone negative feedback. Experimental protocol (top portion): knife cuts were performed in OVX ewes and, after recovery, blood samples were collected (boxes) before, and 7 days after, sc insertion of a progesterone implants (horizontal bar). On day 7, the EOP antagonist, WIN 44,441-3 (WIN), was injected iv at 3 h. Bottom panels depict LH pulse patterns (left) in represent control (top panel) and knife cut (bottom panel) ewes. Bars on the right depict mean (+SEM) LH pulse frequency in OVX (open bars), OVX+P (lightly shaded bar), and OVX+P+WIN (darkly shaded bar) ewes. *$P<0.05$ vs. OVX. Data redrawn from Whisnant and Goodman 1994.

trol animals who had no endogenous LH pulses. Importantly, this increase in Fos expression was limited to the MBH, no Fos was observed in GnRH perikarya in the POA or other areas in the rostral diencephalon. These data thus suggest that interactions between EOP and GnRH neurons within the MBH may be sufficient to account for the inhibition of GnRH pulse frequency by progesterone during the luteal phase.

NEURAL SYSTEMS MEDIATING ESTRADIOL NEGATIVE FEEDBACK DURING THE BREEDING SEASON

The neural systems mediating E_2 inhibition of GnRH pulse amplitude have not been as extensively study as those involved in progesterone negative feedback. Since E_2 clearly has inhibitory effects at the anterior pituitary (Clarke and Cummins 1984, Goodman 1994), it has not been clear whether its inhibition of LH pulse amplitude in breeding season ewes involved actions in the central nervous system. However, recent studies measuring GnRH in hypophyseal portal blood have demonstrated that E_2 does suppress the amount of GnRH released in each pulse (Evans et al. 1994, Goodman et al. 1995b).

A number of studies have reported that EOP antagonists increase LH pulse amplitude in the presence of, but not in the absence of, E_2 and have led to the hypothesis that EOP mediate the negative feedback action of this steroid (Brooks et al. 1986b, Trout and Malven 1987, Whisnant and Goodman 1988, Yang et al. 1988). This hypothesis, however,

remains controversial because stimulatory effects were not always observed, and an indirect index of GnRH secretion was used in all these studies (Trout and Malven 1987, Yang et al. 1988). Therefore, we directly tested this hypothesis by monitoring GnRH pulse patterns before and during infusion of the EOP antagonist, naloxone, in OVX and E_2 -treated OVX (OVX+E) ewes (Goodman et al. 1995b). As illustrated in Fig. 3, naloxone did increase GnRH pulse size in the OVX+E ewes, but this antagonist had an identical effect in the absence of E_2. Unexpectedly, we also observed in this study that naloxone prolonged each GnRH pulse and increased GnRH release between pulses, effects that were also independent of E_2 (Goodman et al. 1995b). Thus, although these results are not consistent with the hypothesis that EOP mediate E_2 negative feedback, they raise the possibility that EOP tone plays an important role in synchronizing the activity of the GnRH neurons driving episodic LH secretion.

Because EOP do not appear to mediate the inhibition of LH pulse amplitude, we have begun to examine other possible neural systems. One possibility, based on previous work (Goodman et al. 1995c), is an α-adrenergic system that acts in the POA. Therefore, we examined the effects of implanting the α-adrenergic antagonist, phenoxybenzamine, into the POA on LH pulse amplitude (Goodman et al. 1996). Local administration of this antagonist to the POA of OVX+E ewes during the breeding season increased LH pulse amplitude, whereas the same treatment in OVX ewes had no effect (Fig. 4). Moreover, a similar stimulatory effect was observed with the α_1-adrenergic receptor antagonist, prazosin (Goodman et al. 1996). It should be noted, however, that the effects of these antagonists were fairly modest. Specifically, only a 50% increase in LH pulse amplitude was observed (Fig. 4), although this may reflect a much more robust increase in GnRH pulse size (Goodman et al. 1995b). Interpretation of these data is further complicated because iv injection of phenoxybenzamine actually decreased LH pulse amplitude in both OVX and OVX+E ewes (Goodman et al. 1996), an effect consistent with earlier studies in the ewe (Jackson 1977, Meyer and Goodman 1986). The difference between the effects of peripheral and central administration of phenoxybenzamine probably reflects multiple sites of action of alpha-adrenergic neurons, some stimulatory and some inhibitory to GnRH release. Similar stimulatory and inhibitory effects have been postulated to occur in the rat (Kalra and Kalra 1983). In light of these caveats, further work is clearly needed to adequately test the hypothesis that α-adrenergic neurons mediate the inhibition of GnRH pulse amplitude by E_2.

Finally, as noted above, E_2 also increases LH pulse frequency in the breeding season (Karsch et al. 1983), and a similar stimulatory effect of this steroid has recently been observed on GnRH pulse frequency (Evans et al. 1994, Goodman et al. 1995b). However, the neural mechanisms responsible for this effect are completely unknown. Specifically, neither naloxone (Goodman et al. 1995b) nor α-adrenergic antagonists (Fig. 4, Goodman et al. 1996) altered GnRH or LH pulse frequency in OVX+E ewes. Thus both the physiological signific-

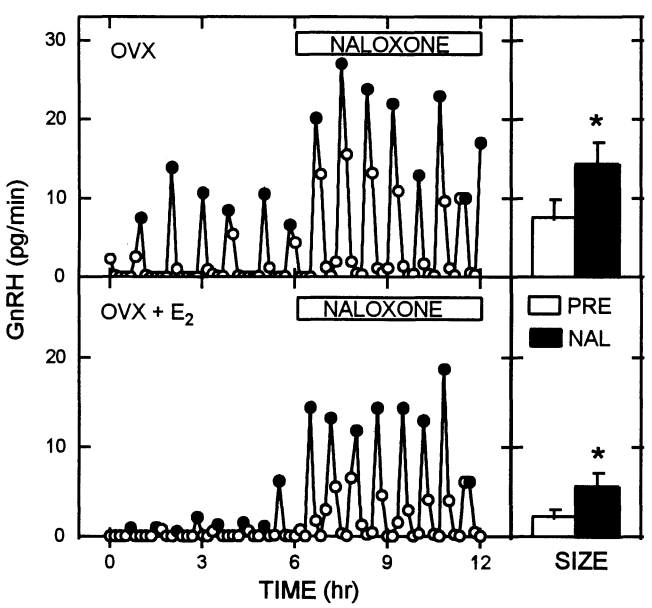

Fig. 3. Effect of naloxone (horizontal bar) on GnRH pulse patterns (left panels) and GnRH pulse size (right panels) in OVX (top panels) and OVX+E (bottom panels) ewes. Open bars are pre-treatment; solid bars during naloxone treatment. *$P<0.05$ vs. pre-treatment. Redrawn from Goodman et al. 1995b.

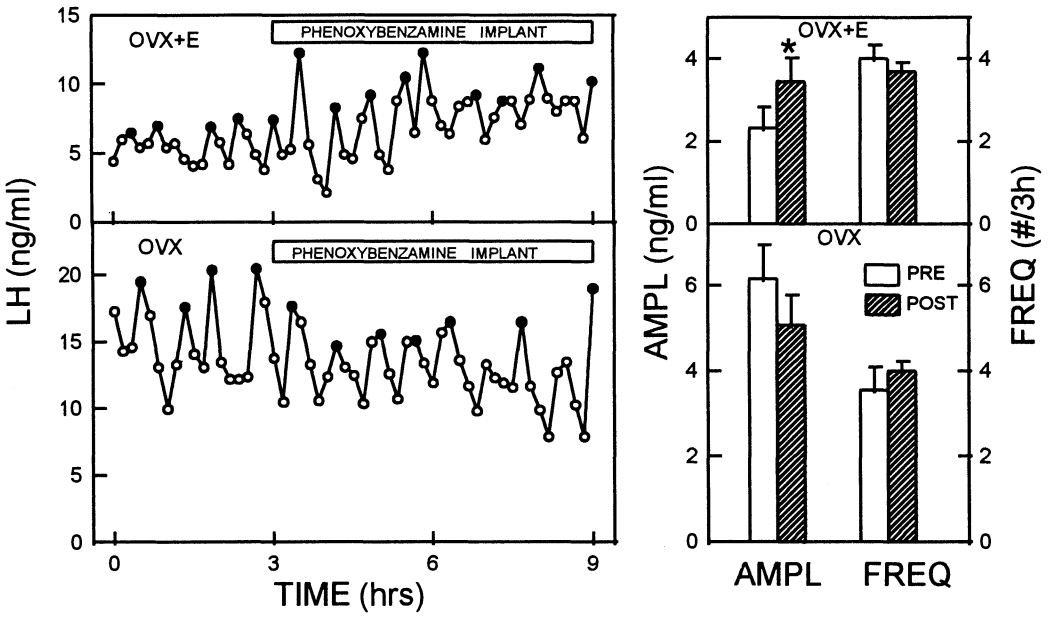

Fig. 4. Effect of implantation of an α-adrenergic antagonist into the POA (horizontal bars) of OVX (bottom panels) and OVX+E (top panels) breeding season ewes. Panels on left are representative LH pulse patterns; bars on the right depict mean (+SEM) LH pulse amplitude (AMPL) and frequency (FREQ) before (open bars), and after (shaded bars), insertion of implants. *$P<0.05$ vs. pre-treatment value. Reprinted from Goodman et al. 1996.

ance and the neural systems involved in this action of E_2 remain obscure.

NEURAL SYSTEMS MEDIATING ESTRADIOL NEGATIVE FEEDBACK IN ANESTRUS

The neural systems responsible for E_2 negative feedback in anestrous ewes are postulated to have an unusual characteristic, in addition to being responsive to E_2 and inhibiting GnRH pulse frequency. Namely, it has been proposed that they are controlled by photoperiod so that they are active in anestrus, but not functional during the breeding season (Goodman and Meyer 1984). The seasonal variation in the functioning of this system accounts for the seasonal alteration in the ability of E_2 to inhibit LH pulse frequency (Fig. 1). Despite this complicating factor (or perhaps because of it) more is know about the system mediating E_2 negative feedback in anestrous ewes than the other neural systems described previously. It should also be noted that since this system inhibits LH pulse frequency, it has been assumed that it affects GnRH pulse frequency, an assumption that has recently been validated by direct measurement of GnRH in hypophyseal portal blood (Barrell al. 1992).

The initial step in identifying the neural systems involved in E_2 negative feedback in anestrus, was an examination of the effects of a number of neurotransmitter receptor antagonists on LH secretion (Meyer and Goodman 1985). In this study, the dopaminergic (DA) antagonist, pimozide (PIM), and the α-adrenergic (NE) antagonist, phenoxybenzamine, increased LH pulse frequency in ovary-intact anestrous ewes, but antagonists to six other receptors did not. This led to the hypothesis that DA and/or NE neurons are holding LH pulse frequency in check in ovary-intact anestrous ewes. Since these antagonists did not alter LH secretion during the breeding season or in OVX ewes during anestrus, we inferred that these catecholaminergic neurons may be responsible for the seasonal variation in the ability of E_2 to inhibit GnRH pulse frequency (Meyer and Goodman 1985, 1986). Based on peripheral administration of DA and NE agonists and antagonists, it was suggested that these systems are

organized "in series", with the NE neurons stimulating a group of DA neurons, which then inhibits GnRH pulse frequency (Goodman 1989).

We next examined where in the hypothalamus these two systems were acting by determining the effects of local administration of these antagonists to ovary-intact ewes. The results of this study suggested that NE acted in the POA, while DA acted in the MBH, to inhibit GnRH pulse frequency (Havern et al. 1991). Taken together the latter two studies led to the prediction that local administration of NE to the POA should inhibit LH pulse frequency, and that this effect should be blocked by PIM placed in the MBH. To test this prediction, drugs were implanted in the appropriate area *via* guide tubes chronically placed in OVX anestrous ewes and LH pulses monitored for 3 h before and 6 h after implantation. NE alone, PIM alone, and NE+PIM were implanted in 9 ewes using a Latin square design.

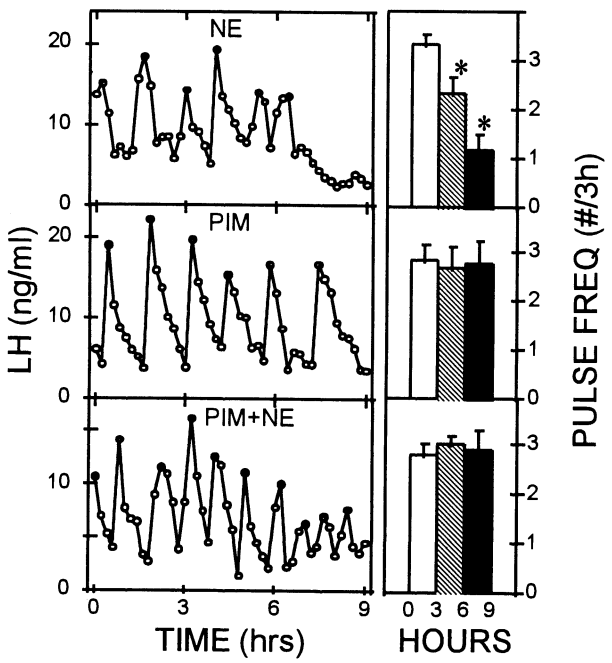

Fig. 5. Effect of implantation of NE in the POA (top panels), PIM in the MBH (middle panels), and PIM+NE (bottom panels) on LH pulse patterns (left) and LH pulse frequency (right) in OVX anestrous ewes. Bars on right depict mean ($n = 9$) LH pulse frequency for the 3 h before and 6 h after NE implantation, divided into 3 h bins. PIM was implanted 24 min before NE; controls received blank implants plus NE or PIM. *$P<0.05$ vs. first 3 h.

Implantation of NE into the POA inhibited LH pulse frequency in 6 of the 9 ewes, and pulse frequency was significantly decreased in this group (Fig. 5). The variability in the effects of NE could largely be accounted for by the site of implantation; most of the effective sites were more ventral (within 2 mm of the optic chiasm) than the ineffective sites. PIM implants alone in the MBH had no effect on pulse frequency, but these implants completely blocked the ability of NE to inhibit LH pulse frequency (Fig. 5). Thus these early studies were largely consistent with the hypothesis that both NE and DA neurons mediate E_2 negative feedback in anestrus, with the NE neurons acting in the POA to stimulate a group of DA neurons, which then acts in the MBH to inhibit GnRH pulse frequency.

If this model is correct, the NE component most likely arises from the brain stem because the ovine POA receives extensive NE innervation (Lehman et al. 1988, Tillet and Thibault 1989) from the locus coeruleus (A6/A7 groups) and the A1 group in the ventro-lateral medulla (Tillet et al. 1993). It should be noted, however, that recent work has called into question a role for NE in mediating E_2 negative feedback in anestrus. First, administration of NE to the POA of anestrous ewes can have stimulatory effects on LH secretion (Scott et al. 1992). Second, peripheral E_2 administration decreased NE levels in the POA of OVX anestrous ewes, although the steroid may also have increased the episodic release of this neurotransmitter (Goodman et al. 1995c). Thus, at this time the importance of NE neurons to E_2 inhibition of GnRH pulse frequency in anestrus is unclear.

In contrast, most recent work has confirmed an inhibitory role for DA, acting via D_2 receptors, during anestrus (Curlewis et al. 1991a, Tilbrook and Clarke 1992, LeCorre and Chemineau 1993a), although there is some conflicting data (Kao et al. 1992). In light of the strong evidence implicating DA in E_2 negative feedback during anestrus, workers began to determine which specific DA neurons are involved in this system. Early lesion studies had suggested that structures in the retrochiasmatic (RCh) area exert an inhibitory effect on reproduc-

tive function during anestrus since lesions in this area induced ovulatory cycles in anestrous ewes (Przekop 1978, Pau et al. 1982). Martin and Thiery also (1987) observed that multiunit electrical activity in the lateral RCh area decreased before an endogenous LH pulse, and electrical stimulation of this area inhibited LH secretion in OVX ewes. Since the lateral RCh contains DA perikarya (the A15 DA group) in the ewe (Lehman et al. 1988, Tillet and Thibault 1989, Tillet et al. 1990), Thiery et al. (1989) postulated that these DA neurons mediate E_2 negative feedback in anestrous ewes. Consistent with this hypothesis, the injection of the neurotoxin, 6-hydroxy-dopamine, into the region of the A15 decreased (but did not completely block) the ability of E_2 to inhibit LH pulse frequency in anestrous ewes (Thiery et al. 1989). However, the neurotoxin did not significantly alter the number of DA neurons in the A15 in this study.

We therefore extended this study, using radiofrequency lesions in either the A15 or the ventromedial portion of the A14 (a group of DA neurons located in the POA, just dorsal to the optic chiasm). These lesions decreased the number of DA perikarya by 50 to 65% in the A14 and A15, respectively (Havern et al. 1994), and, as illustrated in Fig. 6, partially blocked the ability of E_2 to inhibit, and PIM to stimulate, LH pulse frequency in OVX anestrous ewes. In contrast, these lesions had no effect on the ability of E_2 to inhibit LH pulse amplitude during the breeding season (Havern et al. 1994). These results strongly suggest that cells in both the A14 and A15 play a role in the inhibition of GnRH pulse frequency by E_2 in anestrus and that seasonal shifts in the activity of these DA neurons may be responsible for the seasonal variation in response to E_2 negative feedback.

While these neurotoxic and radiofrequency lesions have implicated the A14 and A15 DA neurons in E_2 negative feedback in anestrus, they do not provide any evidence on whether E_2 stimulates these neurons or these neurons act on another system that is responsive to E_2. Therefore, we determined if E_2 could stimulate these DA neurons, using Fos as an

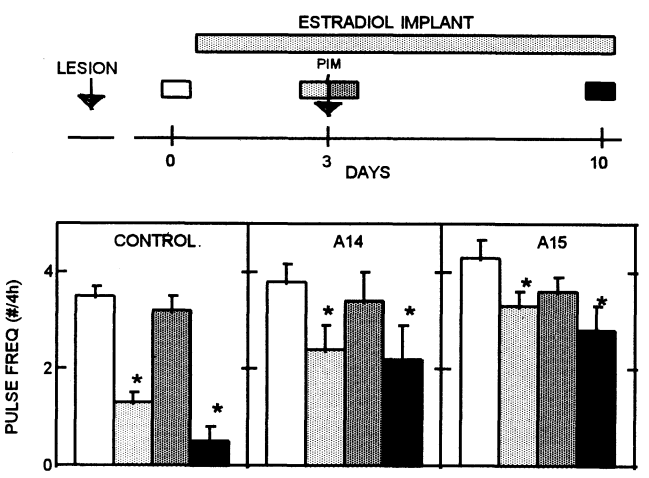

Fig. 6. Effect of lesions in the A14 or A15 areas on E_2 negative feedback in anestrous ewes. Experimental protocol (top portion): lesions (or sham lesions in controls) were done in OVX ewes and, after recovery, blood samples were collected (boxes) before, and 3 and 10 days after, insertion of E_2 implants sc. PIM was inject iv on day 3. Bars depict mean LH pulse frequency before E_2 (open); on day 3 of E_2 treatment, before PIM (lightly shaded) and after PIM (darkly shaded); and on day 10 (black) of E_2 treatment. *$P<0.05$ vs. pre-treatment values. Redrawn from Havern et al. 1994.

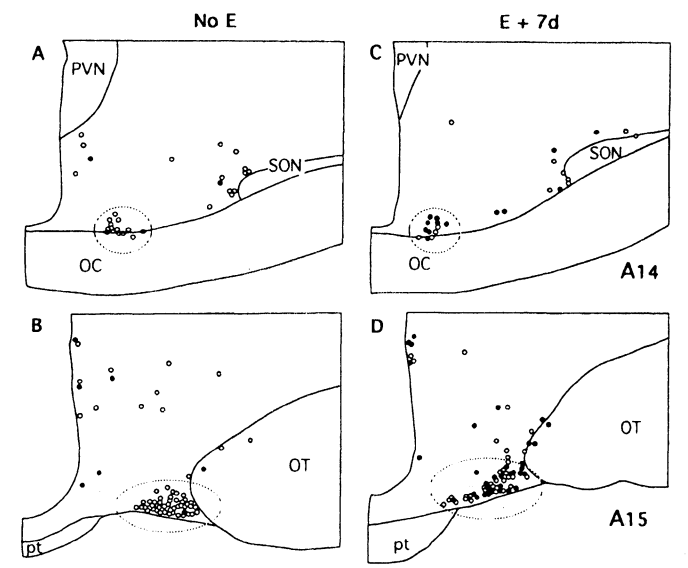

Fig. 7. Camera lucida drawing of coronal sections in the region of the A14 (top sections) and A15 (bottom sections) of an untreated OVX anestrous ewe (sections A,B) and an OVX anestrous ewe treated with E_2 for 7 days (sections C,D). Open circles represent TH-positive neurons without Fos; closed circles,TH-positive neurons containing Fos. Dotted ovals depict areas lesioned in previous experiment. Reprinted from Lehman et al. 1996.

index of neuron activation (Lehman et al. 1996). The expression of Fos in DA neurons was monitored in OVX and OVX+E ewes in both seasons, using a dual immunocytochemical procedure for Fos and tyrosine hydroxylase (TH), the rate limiting enzyme in catecholamine biosynthesis. As expected, E_2 treatment suppressed LH pulse frequency in anestrous, but not breeding season, ewes (Lehman et al. 1996). This action in anestrus was associated with an increase in Fos expression in both the A14 and A15 areas (Figs. 7-8). This increase in Fos was not seen in other DA cell groups in anestrus, and was not observed in any DA neurons in the breeding season (Fig 8). The functional significance of the E_2 -induced increase in Fos is unclear. However, since Fos (in combination with another early intermediate gene product, Jun) can control the TH gene (Icard-Liepkalns et al. 1993), it could be a mechanism for the E_2 -induced increase in TH bioactivity that occurs in the A15 area of anestrous ewes (Gayrard et al. 1994). Regardless of the biological role of Fos, these data provide strong support for the hypothesis that DA neurons in the A14 and A15 areas mediate E_2 negative feedback in anestrus and that there is a seasonal change in the responsiveness of these neurons to E_2.

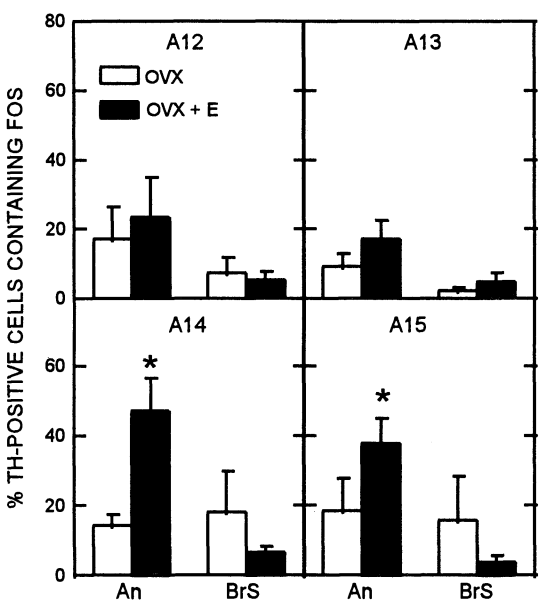

Fig. 8. Percentage of TH-positive cells containing Fos in 4 hypothalamic areas (A12, A13, A14, A15) in OVX (open bars) and OVX+E (black bars) ewes. Data from anestrus (An) and breeding season (BrS) are shown. *$P<0.05$ vs. OVX group. Redrawn from Lehman et al. 1996.

Several studies have attempted to determine where these DA neurons act to inhibit GnRH release. Although close contacts between catecholamine terminals and GnRH cell bodies are observed in the preoptic area (POA) of the ewe (Lehman et al. 1988, Tillet et al. 1989), this input does not appear to come from the A14 or A15 (Tillet et al. 1993). In addition, as noted above, DA does not appear to act in the POA to hold LH pulse frequency in check in anestrous ewes (Havern et al. 1991). On the other hand, there is good evidence that DA acts in the median eminence (ME) region to inhibit LH pulse frequency. Specifically, local administration to the ME of either PIM (Havern et al. 1991), or the catecholamine synthesis inhibitor, α-methyl-paratyrosine (Viguie et al. 1996), increases pulsatile LH secretion in E_2 -treated anestrous ewes. It should be noted, however, that it is difficult to rule out diffusion to adjacent structures in the MBH after administration of these drugs to the ME (Havern et al. 1991). DA neurons synapse on GnRH terminals in the ME (Kuljis and Advis 1989) and some A15 DA neurons appear to project to the ME (Gayrard et al. 1995), but these projections are not to the external zone of the ME, where GnRH terminals are located. Finally, DA content and the bioactivity of TH are higher in the ME of ewes exposed to inhibitory long days than in those exposed to short days, but these effects are independent of E_2 treatment (Thiery 1991, Viguie et al. 1996). Thus while there is strong evidence that DA perikarya in the A15 (and probably the A14) play an important role in E_2 inhibition of GnRH pulse frequency in the anestrous ewe, the site of action of these inhibitory neurons is less clear.

The mechanisms by which E_2 stimulates the A14 and A15 neurons in anestrus also remains to be determined. Since DA perikarya in these areas do not contain E_2 receptors (Lehman and Karsch 1993, Blache 1994), it seems reasonable to assume that another neural system conveys the E_2 signal to these DA cells. A NE system is one possibility, but E_2

does not increase NE release in the POA (Goodman et al. 1995c) and NE perikarya do not appear to contain E_2 receptors in the ewe (Lehman et al. 1993). There is indirect evidence that neurons in the anterior hypothalamic area (Hileman et al. 1994) or the rostral POA (Adrian et al. 1995) may be involved, but the role of these areas has not been conclusively tested. Thus, although DA neurons in the A14 and A15 appear to play a major role in the seasonal variation in response to E_2 negative feedback in ewes, other components of this system remain to be identified.

CONCLUSION

In summary, while the systems involved in E_2 negative feedback in the breeding season remain to be firmly established, those involved in other negative feedback actions are beginning to be identified. Specifically, there is strong evidence that EOP neurons mediate progesterone negative feedback in the breeding season and that DA neurons in the A14 and A15 are responsible for E_2 negative feedback in anestrus. It is interesting to note that EOP and catecholaminergic neurons may also be involved in control of the preovulatory GnRH surge (Domanski et al. 1991, Robinson et al. 1991, Conover et al. 1993). Whether these are same or different neurons remains to be determined, but given the marked differences in the neuroendocrine control of tonic and surge secretion of GnRH it seems likely that different neural systems regulate them. It is also important to recognize that the work reviewed here does not rule out a role for other neural systems in mediating steroid negative feedback. There is some evidence that gamma-aminobutyric acid (GABA) may also be involved: GABA agonists inhibit episodic LH secretion in the ewe (Scott and Clarke 1993a,b); GABA neurons contain E_2 receptors in the ovine hypothalamus (Herbison et al. 1993); and E_2 and progesterone can increase GABA release in the POA (Robinson et al.1991, Robinson and Kendrick 1992). However a direct link between GABA and steroid negative feedback has not yet been demonstrated. There is also some evidence that serotonin may be involved in E_2 negative feedback in anestrous ewes (Gayrard et al. 1992, LeCorre and Chemineau 1993a,b), but the inhibitory effects of this indoleamine in anestrus may be independent of E_2 (Meyer and Goodman 1986, Whisnant and Goodman 1990). Finally, the role of a myriad of other neural systems (e.g., neuropeptide Y, acetylcholine, NO) remain to be examined in the ewe.

Clearly much more work is needed before we understand the mechanisms by which ovarian steroids control pulsatile GnRH secretion. For example, the neural circuitry involved in this control remains unclear. Indeed, the specific GnRH neurons responsible for tonic gonadotropin secretion have yet to be identified. Nevertheless, it is equally clear that considerable progress has been made in identifying the systems involved in the negative feedback actions of E_2 and progesterone in the ewe, progress that will enable us to address these important questions in the future.

ACKNOWLEDGEMENTS

Much of the work reported here represents the collaborative efforts of Drs. Brian Adrian, Bob Havern, Mike Lehman, and Scott Whisnant and I gratefully thank them for their efforts over the years. The technical assistance of Steve Hardy, Jackie Horvath, Shelley Jones, and Avonell Painter has also been critical to the completion of these studies. All the work from my laboratory has been supported by a grant from NIH (HD-17864).

REFERENCES

Adrian B.A., Jansen H.T., Lehman M.N., Goodman R.L. (1995) Estradiol increases Fos expression in a subset of estrogen receptor-containing neurons in the preoptic area. Soc. Neurosci. Abstr. 21: 1891.

Barrell G.K., Moenter S.M., Caraty A., Karsch F.J. (1992) Seasonal changes of gonadotropin releasing hromone secretion in the ewe. Biol. Reprod. 46: 1130-1135.

Blache D., Batailler M., Fabre-Nys C. (1994) Oestrogen receptors in the preoptico-hypothalamic continuum: Immunohistochemical study of the distribution and cell density during induced oestrous cycle in the ovariectomized ewe. J. Neuroendocrinol. 6: 329-339.

Brooks A.N., Haynes N.B., Yang K., Lamming G.E. (1986a) Ovarian steroid involvement in endogenous opioid modu-

lation of LH secretion in seasonally anoestrous mature ewes. J. Reprod. Fertil. 76: 709-715.

Brooks A.N., Lamming G.E., Lees P.D., Haynes N.B. (1986b) Opioid modulation of LH secretion in the ewe. J. Reprod. Fertil. 76: 693-708.

Clarke I.J., Cummins J.T. (1984) Direct pituitary effects of estrogen and progesterone on gonadotropin secretion in the ovariectomized ewe. Neuroendocrinology 39: 267-274.

Clarke I.J., Thomas G.B., Yao B., Cummins J.T. (1987) GnRH secretion throughout the ovine estrous cycle. Neuroendocrinology 46:.82-88.

Conover C.D., Kuljis R.O., Rabii J., Advis J.P. (1993) Beta-endorphin regulation of luteinizing hormone-releasing hormone release at the median eminence in ewes: immunocytochemical and physiological evidence. Neuroendocrinology 57: 1182-1195.

Curlewis J.D., Naylor A.M., McNeilly A.S. (1991a) Evaluation of a possible role for the dopamine D1 and D2 receptors in the steroid dependent suppression of luteinizing hormone secretion in the seasonally anoestrous ewe. J. Neuroendocrinol. 3: 387-391.

Curlewis J.D., Naylor A.M., Rhind S.M., McNeilly A.S. (1991b) Effects of beta-endorphin on pulsatile luteinizing hormone and prolactin secretion during the follicular phase in the ewe. J. Neuroendocrinol. 3: 123-126.

Domanski E., Chomicka L.K., Ostrowska A., Gajewska A., Mateusiak K. (1991) Release of luteinizing hormone-releasing hormone, β-endorphin and noradrenaline by the nucleus infundibularis/median eminence during the periovulatory period in the sheep. Neuroendocrinology 54: 151-158.

Dragunow M., Faull R. (1989) The use of c-fos as a metabolic marker in neuronal pathway tracing. J. Neurosci. Meth. 29: 261-265.

Evans N.P., Dahl G.E., Glover B.H., Karsch F.J. (1994) Central regulation of pulsatile gonadotropin-releasing hormone (GnRH) secretion by estradiol during the period leading up to the preovulatory GnRH surge in the ewe. Endocrinology 134: 1806-1811.

Gayrard V., Malpaux B., Thiéry J.-C. (1992) Oestradiol increases the extracellular levels of amine metabolites in the ewe hypothalamus during anoestrus: a microdialysis study. J. Endocrinol. 135: 421-430.

Gayrard V., Malpaux B., Tillet Y., Thiéry J.-C. (1994) Estradiol increases tyrosine hydroxylase activity of the A15 nucleus dopaminergic neurons during long days in the ewe. Biol. Reprod. 50: 1168-1177.

Gayrard V., Thiéry J.-C., Thibault J., Tillet Y. (1995) Efferent projections from the retrochiasmatic area to the median eminence and to the pars nervosa of the hypophysis with special reference to the A15 dopaminergic cell group in the sheep. Cell. Tiss. Res. 281:.561-567.

Goodman R.L. (1989) Functional organization of the catecholaminergic neural systems inhibiting luteinizing hormone secretion in anestrous ewes. Neuroendocrinology 50: 406-412.

Goodman R.L. (1994) Neuroendocrine control of the ovine estrous cycle. In: The physiology of reproduction (Eds. E. Knobil and J.D. Neill). (Second edition). Vol. 2. Raven Press, New York, p. 659-709.

Goodman R.L., Berriman S.J., Gu X., Lehman M.N. (1995a) Is a subset of gonadotropin-releasing hormone (GnRH) neurons involved in pulsatile GnRH secretion in the ewe? Soc. Neurosci. Abstr. 20: 638.

Goodman R.L., Bittman E.L., Foster D.L., Karsch F.J. (1982) Alterations in the control of luteinizing hormone pulse frequency underlie the seasonal variation in estradiol negative feedback in the ewe. Biol. Reprod. 27: 580-589.

Goodman R.L., Havern R.L., Whisnant C.S. (1996) Alpha-adrenergic neurons inhibit luteinizing hormone pulse amplitude in breeding season ewes. Biol. Reprod. 54: 380-386.

Goodman R.L., Karsch F.J. (1980) Pulsatile secretion of luteinizing hormone: differential suppression by ovarian steroids. Endocrinology 107: 1286-1290.

Goodman R.L., Karsch F.J. (1981) The hypothalamic pulse generator: a key determinant of reproductive cycles in sheep. In: Biological clocks in seasonal reproductive cycles (Eds. B.K. Follett and D.E. Follett). John Wright and Sons, Bristol, p. 223-236.

Goodman R.L., Meyer S.L. (1984) Effects of pentobarbital anesthesia on tonic luteinizing hormone secretion in the ewe: evidence for active inhibition of luteinizing hormone in anestrus. Biol. Reprod. 30: 374-381.

Goodman R.L., Parfitt D.B., Evans N.P., Dahl G.E., Karsch F.J. (1995b) Endogenous opioid peptides control the amplitude and shape of gonadotropin-releasing hormone pulses in the ewe. Endocrinology 136: 2412-2420

Goodman R.L., Robinson J.E., Kendrick K.M., Dyer R.G. (1995c) Is the inhibitory action of estradiol on luteinizing hormone pulse frequency in anestrous ewes mediated by noradrenergic neurons in the preoptic area? Neuroendocrinology 61: 284-292.

Havern R.L., Whisnant C.S., Goodman R.L. (1991) Hypothalamic sites of catecholamine inhibition of luteinizing hormone in the anestrous ewe. Biol. Reprod. 44: 476-482.

Havern R.L., Whisnant C.S., Goodman R.L. (1994) Dopaminergic structures in the ovine hypothalamus mediating estradiol negative feedback in anestrous ewes. Endocrinology 134: 1905-1914.

Herbison A.E., Robinson J.E., Skinner D.C. (1993) Distribution of estrogen receptor-immunoreactive cells in the preoptic area of the ewe: colocalization with glutamic acid decarboxylase but not luteinizing hormone-releasing hormone. Neuroendocrinology 57: 751-759.

Hileman S.M., Kuehl D.E., Jackson G.L. (1994) Effect of anterior hypothalamic area lesions on photoperiod-induced shifts in reproductive activity in the ewe. Endocrinology 135: 1816-1823.

Horton R.J.E., Cummins J.T., Clarke I.J. (1987) Naloxone evokes large amplitude GnRH pulses in luteal phase ewes. J. Reprod. Fertil. 81: 277-286.

Icard-Liepkalns C., Biguet N.F., Vyas S., Robert J.J., Sassone-Corsi P., Mallet J. (1993) AP-1 complex and c-fos transcription are involved in TPA provoked and trans-synaptic inductions of the tyrosine hydroxylase gene: insights into long-term regulatory mechanisms. J. Neurosci. Res. 32: 290-298.

Jackson G.L. (1977) Effect of adrenergic blocking drugs on secretion of luteinizing hormone in the ovariectomized ewe. Biol. Reprod. 16: 543-548.

Kalra S.P., Kalra P.S. (1983) Neural regulation of luteinizing hormone secretion in the rat. Endocrinol. Rev. 4: 311-351.

Kalra S.P., Kalra P.S. (1989) Do testosterone and estradiol-17β enforce inhibition or stimulation of luteinizing hormone-releasing hormone secretion? Biol. Reprod. 41: 559-570.

Karsch F.J. (1987) Central actions of ovarian steroids in the feedback regulation of pulsatile secretion of luteinizing hormone. Ann. Rev. Physiol. 49: 365-382.

Karsch F.J., Bittman E.L., Foster D.L., Goodman R.L., Legan S.J., Robinson J.E. (1984) Neuroendocrine basis of seasonal reproduction. Recent Prog. Horm. Res. 40: 185-232.

Karsch F.J., Cummins J.T., Thomas G.B., Clarke I.J. (1987) Steroid feedback inhibition of pulsatile secretion of gonadotropin-releasing hormone in the ewe. Biol. Reprod. 36: 1207-1218.

Karsch F.J., Foster D.L., Goodman R.L., Bittman E.L. (1983) A role for estradiol in enhancing luteinizing hormone pulse frequency in the follicular phase of the estrous cycle of sheep. Endocrinology 113: 1333-1339.

Kao C., Schaeffer D.J., Jackson G.L. (1992) Different neuroendocrine systems modulate pulsatile luteinizing hormone secretion in photosuppressed and photorefractory ewes. Biol. Reprod. 46: 425-434.

Kuljis R.O., Advis J.P. (1989) Immunocytochemical and physiological evidence of a synapse between dopamine and luteinizing horomone releasing hormone containing neurons in the ewe median eminence. Endocrinology 124: 1579-1581.

LeCorre S., Chemineau P. (1993a) Control of photoperiodic inhibition of luteinizing hormone secretion by dopaminergic and serotonergic systems in ovariectomized Il-de-France ewes supplemented with oestradiol. J. Reprod. Fertil. 97: 367-373.

Le Corre S., Chemineau P. (1993b) Serotonergic $5HT_2$ receptors mediate the inhibitory action of serotonin on luteinizing hormone secretion in ovariectomized, estradiol-treated ewes that are refractory to short days. Biol. Reprod. 49: 140-147.

Legan S.J., Karsch F.J., Foster D.L. (1977) The endocrine control of seasonal reproductive function in the ewe: a marked change in response to the negative feedback action of estradiol on luteinizing hormone secretion. Endocrinology 101: 818-824.

Lehman M.N., Durham D.M., Jansen H.T., Adrian B.A., Goodman R.L. (1996) Dopaminergic A14/A15 neurons are activated during estradiol negative feedback in the anestrous ewe. Endocrinology (in press)

Lehman M.N., Ebling F.J.P., Moenter S.M., Karsch F.J. (1993) Distribution of estrogen receptor-immunoreactive cells in the sheep brain. Endocrinology 133: 876-886.

Lehman M.N., Karsch F.J. (1993) Do gonadotropin-releasing hormone, tyrosine hydroxylase-, and β-endorphin-immunoreactive neurons contain estrogen receptors? A double-label immunocytochemical study in the Suffolk ewe. Endocrinology 133: 887-895.

Lehman M.N., Karsch F.J., Silverman A.J. (1988) Potential sites of interaction between catecholamines and LHRH in the sheep brain. Brain Res. Bull. 20: 49-58.

Malven P.V., Stanisiewski E.P., Haglof S.A. (1990) Ovine brain areas sensitive to naloxone-induced stimulation of luteinizing hormone release. Neuroendocrinology 52: 373-381.

Martin G.B. (1984) Factors affecting the secretion of luteinizing hormone in the ewe. Biol. Rev. 59: 1-87.

Martin G.B., Scaramuzzi R.J., Henstridge J.D. (1983) Effects of oestradiol, progesterone and androstenedione on the pulsatile secretion of luteinizing hormone in ovariectomized ewes during spring and autumn. J. Endocrinol. 96: 181-193.

Martin G.B., Thiery J.C. (1987) Hypothalamic multiunit activity and LH secretion in conscious sheep. Exp. Brain Res. 67: 469-478.

Meyer S.L., Goodman R.L. (1985) Neurotransmitters involved in mediating the steroid-dependent suppression of pulsatile luteinizing hormone secretion in anestrous ewes: effects of receptor antagonists. Endocrinology 116: 2054-2061.

Meyer S.L., Goodman R.L. (1986) Separate neural systems mediate the steroid-dependent and steroid-independent suppression of tonic luteinizing hormone secretion in the anestrous ewe. Biol. Reprod. 35: 562-571.

Moenter S.M., Brand R.M., Karsch F.J. (1992a) Dynamics of gonadotropin-releasing hormone (GnRH) secretion during the GnRH surge: Insights into the mechanisms of GnRH surge induction. Endocrinology 130: 2978-2984.

Moenter S.M., Brand R.C., Midgley A.R., Karsch F.J. (1992b) Dynamics of gonadotropin-releasing hormone release during a pulse. Endocrinology 130: 503-510.

Moenter S.M., Caraty A., Locatelli A., Karsch F.J. (1991) Pattern of gonadotropin-releasing hormone (GnRH) secretion leading up to ovulation in the ewe: existance of a preovulatory GnRH surge. Endocrinology 129: 1175-1182

Moore C.R., Price O. (1932) Gonad hormone functions and the reciprocal influence between gonads and hypophysis

with its bearing on the problem of sex hormone antagonisms. Am. J. Anat. 50: 13-71.

Nakai Y., Plant T.M., Hess D.L., Keogh E.J., Knobil E. (1978) On the sites of the negative and positive feedback actions of estradiol in the control of gonadotropin secretion in the Rhesus monkey. Endocrinology 102: 1008-1014.

Pau K-Y.F., Kuehl D.E., Jackson G.L. (1982) Effect of frontal hypothalamic deafferentation on luteinizing hormone secretion and seasonal breeding in the ewe. Biol. Reprod. 27: 999-1009.

Przekop F. (1978) Effect of anterior deafferentation of the hypothalamus on the release of luteinizing hormone (LH) and reproductive function in sheep. Acta Physiol. Pol. 29: 393-407.

Robinson J.E., Kendrick K.M. (1992) Inhibition of luteinizing hormone secretion in the ewe by progesterone: associated changes in the release of gamma-aminobutyric acid and noradrenaline in the preoptic area as measured by intracranial microdialysis. J. Neuroendocrinol. 4: 231-236.

Robinson J.E., Kendrick K.M., Lambart C.E. (1991) Changes in the release of gamma-aminobutyric acid and catecholamines in the preoptic/septal area prior to and during the preovulatory surge of luteinizing hormone. J. Neuroendocrinol. 3: 393-399.

Robinson J.E., Radford H.M., Karsch F.J. (1985) Seasonal changes in pulsatile luteinizing hormone (LH) secretion in the ewe: relationship of frequency of LH pulses to day length and response to estradiol negative feedback. Biol. Reprod. 33: 324-334.

Scott C.J., Clarke I.J. (1993a) Inhibition of LH secretion in ovariectomised ewes during the breeding season by γ-aminobutyric acid (GABA) is effected by GABA-A receptors, but not GABA-B receptors. Endocrinology 132: 1789-1796.

Scott C.J.,., Clarke I.J. (1993b) Evidence that changes in the function of the subtypes of the receptors for γ-amino butyric acid may be involved in the seasonal changes in the negative-feedback effects of estrogen on gonadotropin-releasing hormone secretion and plasma luteinizing hormone levels in the ewe. Endocrinology 133: 2904-2912.

Scott C.J., Cummins J.T., Clarke I.J. (1992) Effects on plasma luteinizing hormone (LH) levels of microinjection of noradrenaline and adrenaline into the septo-preoptic area of the brain of the ovariectomized ewe; changes with season and chronic oestrogen treatment. J. Neuroendocrinol. 4: 131-141.

Silverman A.-J., Livne I., Witkin J.W. (1994) The gonadotropin-releasing hormone (GnRH) neuronal systems: immunocytochemistry and in situ hybridization. In: The Physiology of reproduction (Eds. E.Knobil and J.D. Neill). (Second edition). Vol. 1. Raven Press, New York, p. 1683-1709.

Thiéry J.-C. (1991) Monoamine content of the stalk-median eminence and hypothalamus in adult female sheep as affected by daylength. J. Neuroendocrinol. 3: 407-411.

Thiéry J.-C., Martin G.B., Caldani M., Quentin M., Jamain C., Ravault J.P. (1989) Role of hypothalamic catecholamines in the regulation of luteinizing hormone and prolactin secretion in the ewe during seasonal anestrus. Neuroendocrinology 49: 80-87.

Tilbrook A.J., Clarke I.J. (1992) Evidence that dopaminergic neurons are not involved in the negative feedback effect of testosterone on luteinizing hormone in rams in the nor-breeding season. J. Neuroendocrinol. 4: 365-374.

Tillet Y., Batailler M., Kreiger-Poullet M., Thibault J. (1990) Presence of dopamine immunoreactive cell bodies in the catecholaminergic group A15 of the sheep brain. Histochemistry 93: 327-333.

Tillet Y., Batailler M., Thibault J. (1993) Neuronal projections to the medial preoptic area of the sheep, with special reference to monoaminergic afferents: immunohistochemical and retrograde tract tracing studies. J. Comp. Neurol. 330: 195-220.

Tillet Y., Caldani M., Batailler M. (1989) Anatomical relationships of monoaminergic and neuropeptide Y-containing fibers with luteinizing hormone-releasing hormone systems in the preoptic area of the sheep brain: immunohistochemical studies. J. Chem. Neuroanat. 2: 319-326.

Tillet Y., Thibault J. (1989) Catecholamine-containing neurons in the sheep brainstem and diencephalon: immunohistochemical study with tyrosine hydroxylase (TH) and dopamine-ß-hydroxylase (DBH) antibodies. J. Comp. Neurol. 290: 69-104.

Trout W.E., Malven P.V. (1987) Effects of exogenous estradiol-17ß and progesterone on naloxone-reversible inhibition of the release of luteinizing hormone in ewes. J. Anim. Sci. 65: 1602-1609.

Viguie C., Thibault J., Thiery J.-C., Tillet Y., Malpaux B. (1996) Photoperiodic modulation of monoamines and amino-acids involved in the control of prolactin and LH secretion in the ewe: evidence for a regulation of tyrosine hydroxylase activity. J. Neuroendocrinol. (in press)

Viguie C., Thiery J.-C., Picard S., Malpaux B. (1996) Blockade of tyrosine hydroxylase in the median eminence stimulates LH pulsatile secretion in long-day treated ewes. Biol. Reprod. 52 (Suppl. 1): 155.

Weesner G.D., Malven P.V. (1990) Intracerebral immunoneutralization of beta-endrophin and met-enkephalin disinhibits release of pituitary luteinizing hormone in sheep. Neuroendocrionology 52: 382-388.

Whisnant C.S., Curto K., Goodman R.L. (1993) Immunocytochemical localization of beta-endorphin and gonadal steroid regulation of proopiomelanocortin messenger ribonucleic acid in the ewe . Neuroendocrinology 56: 812-821.

Whisnant C.S., Goodman R.L. (1988) Effects of an opioid antagonist on pulsatile luteinizing hormone secretion in the ewe vary with changes in steroid negative feedback. Biol. Reprod. 39: 1032-1038.

Whisnant C.S., Goodman R.L. (1990) Further evidence that serotonin mediates the steroid independent inhibiton of luteinizing hormone secretion in anestrous ewes. Biol. Reprod. 42: 656-661.

Whisnant C.S., Goodman R.L. (1994) Effect of anterior hypothalamic deafferentation on the negative feedback of gonadal steroids on luteinizing hormone pulse frequency in the ewe. Dom. Anim. Endocrinol. 11: 151-159.

Whisnant C.S., Havern R.L., Goodman R.L. (1991) Endogenous opioid suppression of luteinizing hormone pulse frequency and amplitude in the ewe: hypothalamic sites of action. Neuroendocrinology 54: 587-593.

Yang K., Haynes N.B., Lamming G.E., Brooks A.N. (1988) Ovarian steroid hormone involvement in endogenous opioid modulation of LH secretion in mature ewes during the breeding and non breeding seasons. J. Reprod. Fertil. 83: 129-139.

Received 15 April 1996, accepted 15 May 1996