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Feedback actions of estradiol on GnRH secretion during the follicular phase of the estrous cycle

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Abstract. The pattern of GnRH secretion during the follicular phase of the estrous cycle of sheep is characterized by an initial marked change in episodic secretion (increased frequency and decreased amplitude) followed by a massive and sustained discharge – the preovulatory GnRH surge. Studies employing a physiological model for the follicular phase have revealed that estradiol has profound and complex feedback effects on GnRH release during the preovulatory period. These include both quantitative effects on pulses (stimulation of frequency, inhibition of amplitude) and qualitative effects (altering pulse shape, stimulating interpulse secretion), in addition to inductng a preovulatory GnRH surge. In stimulating the surge, estradiol causes a highly characteristic change in the minute-to-minute pattern of GnRH in hypophyseal portal blood. Initially, a strictly episodic pattern gives way to one in which GnRH is consistently elevated between pulses. Then, following enhancement of both pulsatile and interpulse components, GnRH becomes extremely high and variable for the majority of the surge. From this point, a regular and well organized pulse pattern is not apparent. The characteristic time course of GnRH at surge onset provides insight into possible mechanistic changes in the GnRH neurosecretory system. Such changes include quantitative and qualitative alterations in the pulse generating mechanism, recruitment of a surge specific population of GnRH neurones, morphologic alterations in GnRH neurones and neighboring cells, and changes in efficiency or route of delivery of GnRH from its site of release to the portal vasculature. These possibilities, while untested and speculative, provide a conceptual framework for future research.

Mini-review

Key words: positive feedback, negative feedback, LH surge, GnRH surge, LH pulses, GnRH pulses, ovulation

INTRODUCTION

The development of techniques for hypophyseal portal blood collection from conscious undisturbed sheep (Clarke and Cummins 1982, Caraty et al. 1994) has enabled detailed characterization of gonadotropin-releasing hormone (GnRH) secretory patterns leading to ovulation (Clarke et al. 1987, Moenter et al. 1991, Evans et al. 1995b). Such studies have revealed marked changes in GnRH secretion during the preovulatory period. Fig. 1 depicts the patterns of GnRH and LH we have typically observed during the estrous cycle. Following regression of the corpus luteum, marked changes occur in the episodic pattern of GnRH release, with frequency initially increasing from the luteal phase rate of ~1 pulse/4 h to ~1 pulse/h during the early to mid-follicular phase. As the follicular phase progresses and serum estradiol concentrations rise, GnRH pulse frequency continues to increase and GnRH-pulse amplitude decreases. At the subsequent onset of the preovulatory LH surge, a marked and sustained release of GnRH develops – the preovulatory GnRH surge (Moenter et al. 1991).

Due to the importance of estradiol in the feedback regulation of gonadotropin secretion, its role in regulating GnRH secretion during the preovulatory period has been investigated extensively. For this purpose, we have employed an endocrine model for the follicular phase, one in which physiological concentrations of circulating estradiol and progesterone are restored in their naturally occurring temporal sequence in the absence of the ovaries (Goodman et al. 1981). Studies utilizing this artificial follicular phase model indicate estradiol has profound feedback actions on GnRH secretion during the preovulatory period. These feedback actions are highly complex and include an influence on

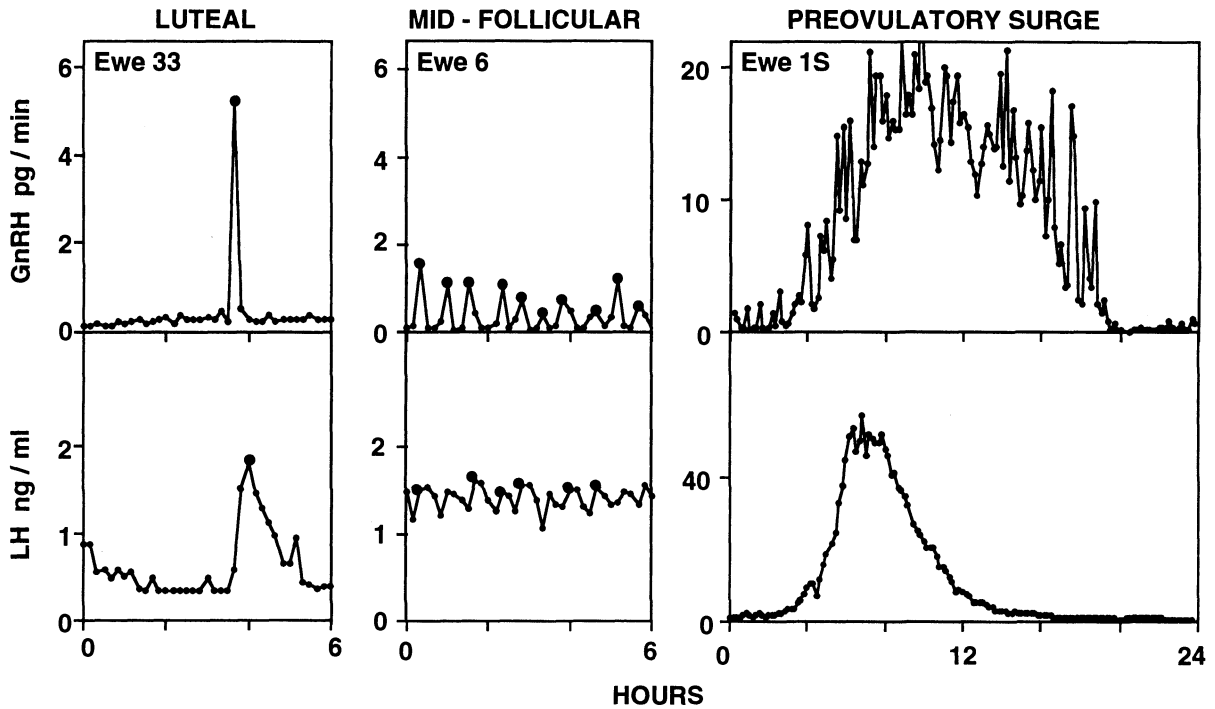


Fig. 1. Representative patterns of GnRH in hypophyseal portal blood (top) and LH in peripheral blood (bottom) of ewes at selected estrous cycle stages: luteal phase (left), mid-follicular phase (middle), preovulatory surge (right). Samples were obtained at 10-min intervals for either 6 h (luteal and mid-follicular phases) or 24 h (preovulatory surge). Large circles depict peaks of statistically identified pulses; formal pulse analysis was not performed for the preovulatory surge. Note different scales along both axes for the preovulatory surge. Modified from Moenter et al. (1991), Barrell et al. (1992), and Evans and Karsch (1995).

GnRH pulse frequency, amplitude and shape, enhancement of interpulse secretion, and induction of the preovulatory GnRH surge. This report briefly summarizes these findings and considers them in light of possible mechanisms by which a rise in circulating estradiol might induce the preovulatory GnRH surge.

EFFECTS OF ESTRADIOL PRIOR TO ONSET OF THE GnRH SURGE

To investigate the role of estradiol in regulating the GnRH secretory pattern leading into the preovulatory GnRH surge, we employed the artificial follicular phase model as illustrated in Fig. 2. Following the withdrawal of progesterone (–P), ewes were allocated to three groups according to their estradiol treatment: (1) no estradiol group (NO E) – estradiol treatment was terminated at the time of progesterone withdrawal; (2) basal estradiol group (Basal E) – a low level of circulating estradiol typical of the luteal phase was maintained by a small Silastic estradiol implant; (3) incremental estradiol group (INC E) – circulating estradiol was increased from a luteal phase level to a peak follicular phase level by stepwise addition of estradiol implants. Initially, portal blood was collected every 10 min for 20 h to assess pulsatile GnRH release. Jugular blood was sampled at the same frequency to monitor the LH pulse pattern. Sampling frequency for portal blood was then increased to 1 min for 3 additional h to obtain a more detailed analysis of moment-to-moment GnRH release. The GnRH/LH surge was expected only in ewes given the incremental estradiol treatment; portal blood collection ended 2-6 hours before this surge began (black box in Fig. 2).

GnRH patterns observed in 10-min samples (initial 20 h) revealed estradiol influenced pulsatile GnRH release in a dose-dependent fashion (results summarized in Fig. 3; full details in Evans et al. 1994). In particular, estradiol significantly reduced GnRH pulse size, increased pulse frequency, and inhibited the total amount of GnRH released. These effects were more pronounced in ewes receiving the higher estradiol concentrations. The step-wise rise of estradiol in the incremental group was associated with a progressive decrease in total GnRH release after a delay of approximately 6 h. In general, LH

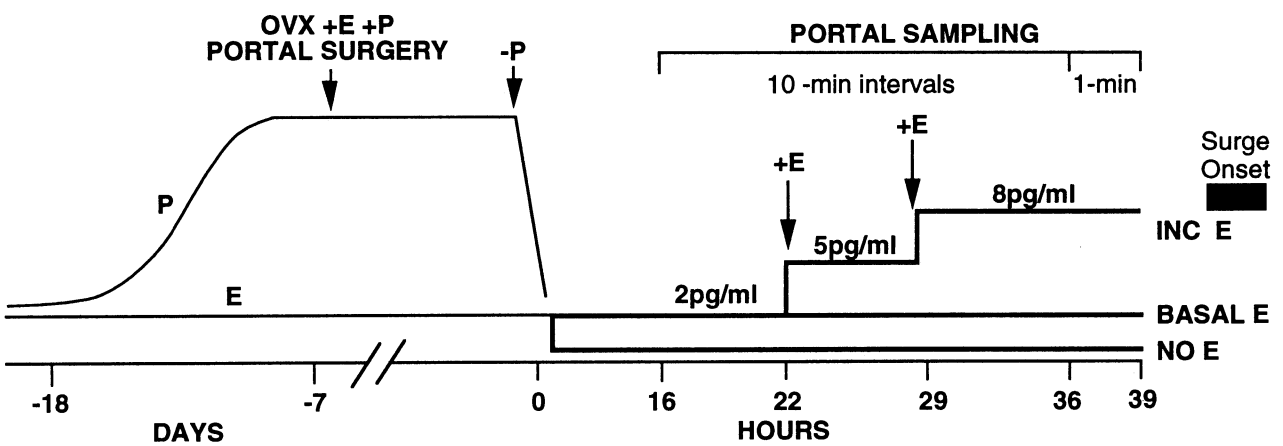


Fig. 2. Experimental design using artificial follicular phase model to investigate estradiol feedback regulation of GnRH secretion during the pre-surge period of the ewe. During the mid-luteal phase of the cycle, ewes were ovariectomized (OVX), prepared surgically for subsequent portal blood collection and treated with Silastic implants to maintain physiological circulating levels of estradiol (E) and progesterone (P). One week later, the progesterone implants were removed (-P at hour 0) and estradiol was manipulated to produce 3 groups: no E, basal E, incremental (INC) E (see text for details of treatments). Approximate serum estradiol concentrations produced by these treatments are indicated as pg/ml. Samples of hypophyseal portal and jugular blood were obtained at 10-min intervals from hour 16 to 36, after which frequency of portal sampling was increased to 1 min until hour 39. Black box at the far right indicates range of LH surge onsets in the incremental E group, as determined in jugular samples. Modified from Evans et al. (1994).

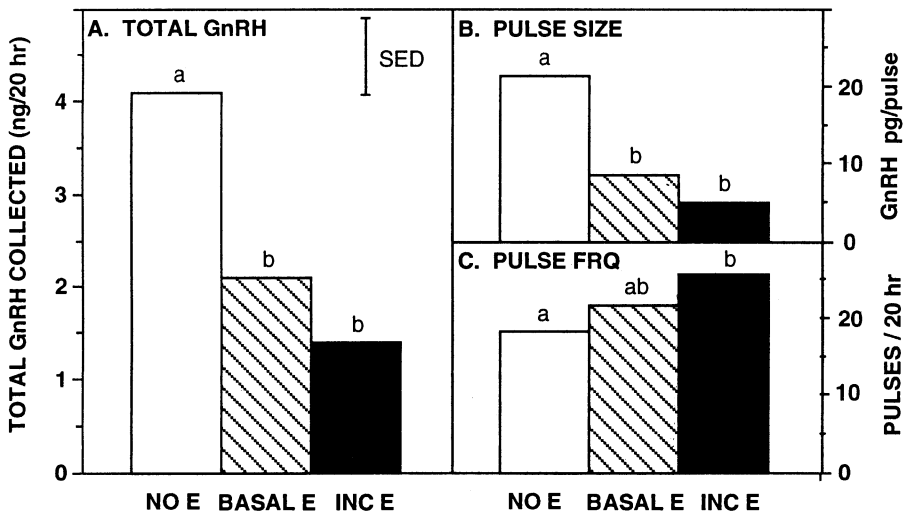
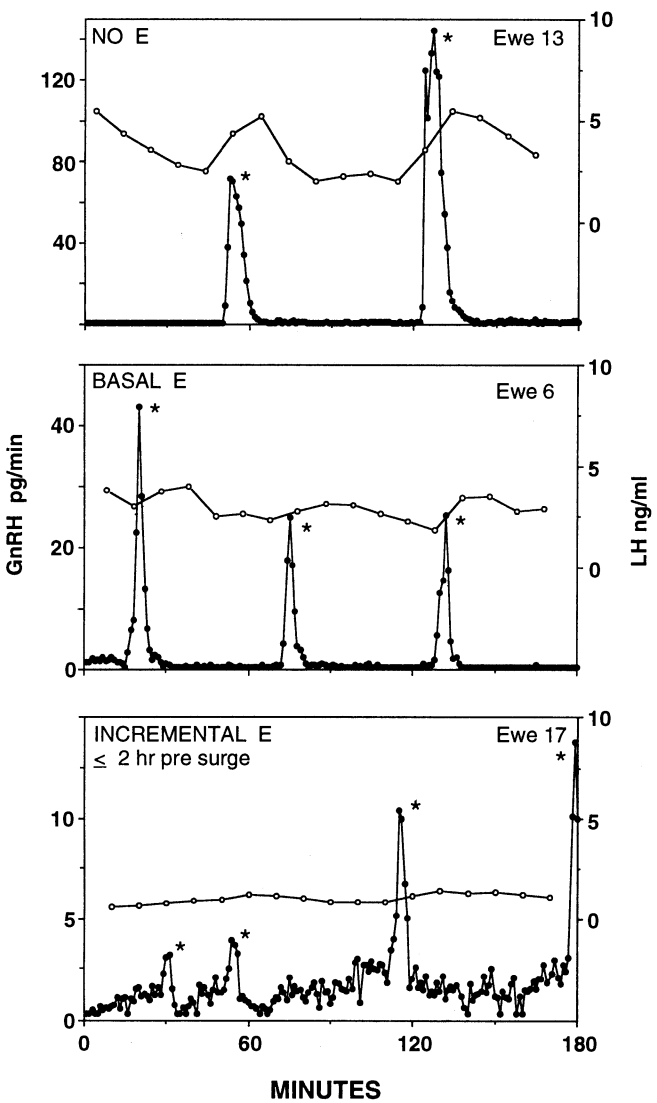


Fig. 3. Effect of manipulating estradiol (E) in the artificial follicular phase model on three aspects of GnRH secretion during 20 h of sampling hypophyseal portal blood at 10-min intervals: total GnRH (panel A), GnRH pulse size (panel B), GnRH pulse frequency (panel C). Bars depict mean values (5 ewes/group) for the no E, basal E and incremental (Inc) E groups (treatments described in Fig. 2). Different letters signify group differences ($P \leq 0.05$); SED bar indicates the standard error of group differences as calculated from analysis of variance. Modified from Evans et al. (1994).



patterns in peripheral blood changed in parallel fashion, although the inhibition of LH occurred prior to that for GnRH (details in Evans et al. 1994).

More detailed analysis of the GnRH pattern obtained by 1-min sampling reinforced the conclusion that estradiol alters episodic GnRH secretion in a dose-dependent fashion during the presurge period (Evans et al. 1995a). In particular, estradiol reduced GnRH pulse size and increased frequency, both effects being most evident in the incremental estradiol group (Fig. 4). In addition, this analysis revealed two qualitaive effects of estradiol not evident from the less frequent sampling. First, estradiol enhanced GnRH secretion between pulses. This effect was most evident in the incremental estradiol group (Fig. 4, lower panel). Second, estradiol significantly altered GnRH pulse shape, reducing the rate of rise and to a lesser extent the rate of fall. As a result, GnRH pulses appeared more triangular in shape in estradiol-treated ewes compared to the more

Fig. 4. Representative GnRH patterns (closed circles) in 1-min samples of hypophyseal portal blood and LH (open circles) in 10-min samples of peripheral blood of ewes treated with no estradiol (top), basal estradiol (middle) or incremental estradiol (bottom) in the artificial follicular phase model (see Fig. 2 for treatment details.) An LH surge began within 2 h after portal sampling ended in ewes receiving the incremental estradiol treatment. Statistically identified peaks of GnRH pulses are indicated by asterisks (*). Modified from Evans et al. (1995a).

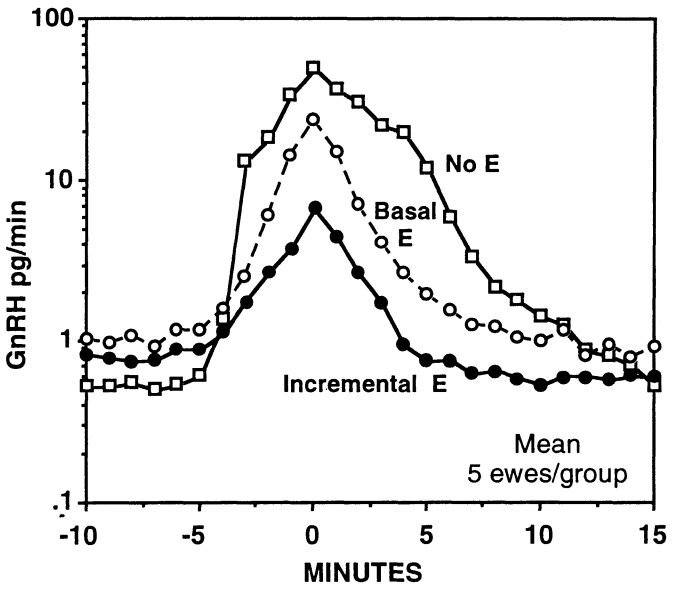


Fig. 5. Effect of estradiol (E) on GnRH pulse shape during the pre-surge period in the artificial follicular phase model. A mean GnRH pulse profile (centred around peaks) was determined for each animal, and the average for all animals in the no E, basal E, and incremental E groups was determined to derive the plots (5 ewes/group). Data were obtained from hypophyseal portal blood sampled at 1-minute intervals; see Fig. 2 for treatment details. Modified from Evans et al. (1995a).

square-wave pattern in the no estradiol group (Fig. 5). Again these effects were dose-dependent, being most evident in animals receiving the incremental estradiol treatment.

The foregoing studies suggest estradiol exerts both quantitative and qualitative feedback effects on GnRH secretion during the presurge period in the ewe. These effects are stimulatory as well as inhibitory, with estradiol enhancing both pulse frequency and secretion between pulses but reducing both pulse size and total amount of GnRH released. Additionally, estradiol appeared to alter GnRH pulse shape. We find it somewhat difficult and perhaps not useful to regard these various effects as positive and/or negative feedback because, in the same animal and at the same time, estradiol enhances some aspects of GnRH release while inhibiting others. Rather, we consider it more constructive to conclude that estradiol modifies the operational characteristics of the GnRH neurosecretory system during the presurge period. This prompted us to consider the possibility that these changes are involved in generating the preovulatory GnRH surge.

EFFECTS OF ESTRADIOL DURING DEVELOPMENT OF THE GnRH SURGE

The surge-inducing action of estradiol is fascinating when viewed in the context of GnRH secretory patterns in other physiological states. At other times (luteal phase, early follicular phase, following ovariectomy), GnRH secretion is strictly episodic. During the surge, however, GnRH remains continuously elevated in portal blood for as long as 24 h (e.g., Fig. 1). Given this difference, we sought to assess whether this sustained GnRH increase is due to an extreme stimulation of pulse frequency or to the more qualitative changes in secretory pattern seen in the presurge period. For this purpose, we used a slight modification of the artificial follicular phase model described in Fig. 2 and sampled hypophyseal portal blood very frequently (30-s to 2-min intervals) during the surge .

We first focused on GnRH secretory dynamics during the peak and declining limb of the surge. We did not obtain any evidence of a strictly pulsatile pattern in 39 of 40 sampling periods; GnRH values invariably remained far greater than the pre-surge baseline. The one exception was a regular oscillation every 6 min near the end of the surge (Fig. 1 in Moenter et al. 1992b). Our interpretation of those findings was that estradiol induces the surge by altering the mode of operation of the GnRH neurosecretory system in such a way that the GnRH pattern delivered to the pituitary changes from being strictly episodic to continuous. However, another report describing the time course of GnRH in highly frequent samples during the surge was not entirely consistent with this interpretation. In that study, the surge was induced by bolus injection of estradiol benzoate into long-term ovariectomized ewes (Clarke 1993). The results led to the conclusion that GnRH pulse frequency increases during the surge, although no single GnRH pattern prevailed as the surge developed in that animal model.

In view of our finding that estradiol can induce qualitative changes in the time course of GnRH secretion and can regulate pulse frequency and amplitude, we set out to determine how these various effects might relate to genesis of the surge. Using the artificial follicular phase model and high frequency sampling to characterize moment-to-moment changes in GnRH secretion, we focused on the period surrounding onset of the surge.

Hypophyseal portal blood was sampled from 7 ewes at 1-min intervals for 11 h around the time the surge was expected to begin. All 7 ewes exhibited a characteristic change in their GnRH pattern prior to and during development of the surge. Figure 6 depicts results in a representative ewe. Initially, GnRH was strictly episodic, with values usually falling below assay sensitivity between pulses (0-265 min, Stage I in Fig. 6). This was followed by a period lasting 2-4 h when both episodic and non episodic components of GnRH release were observed (265-395 min, Stage II in Fig. 6). During this time, no consistent change occurred in the GnRH pulse pattern but values between pulses became detectable. GnRH pulses then became larger and more

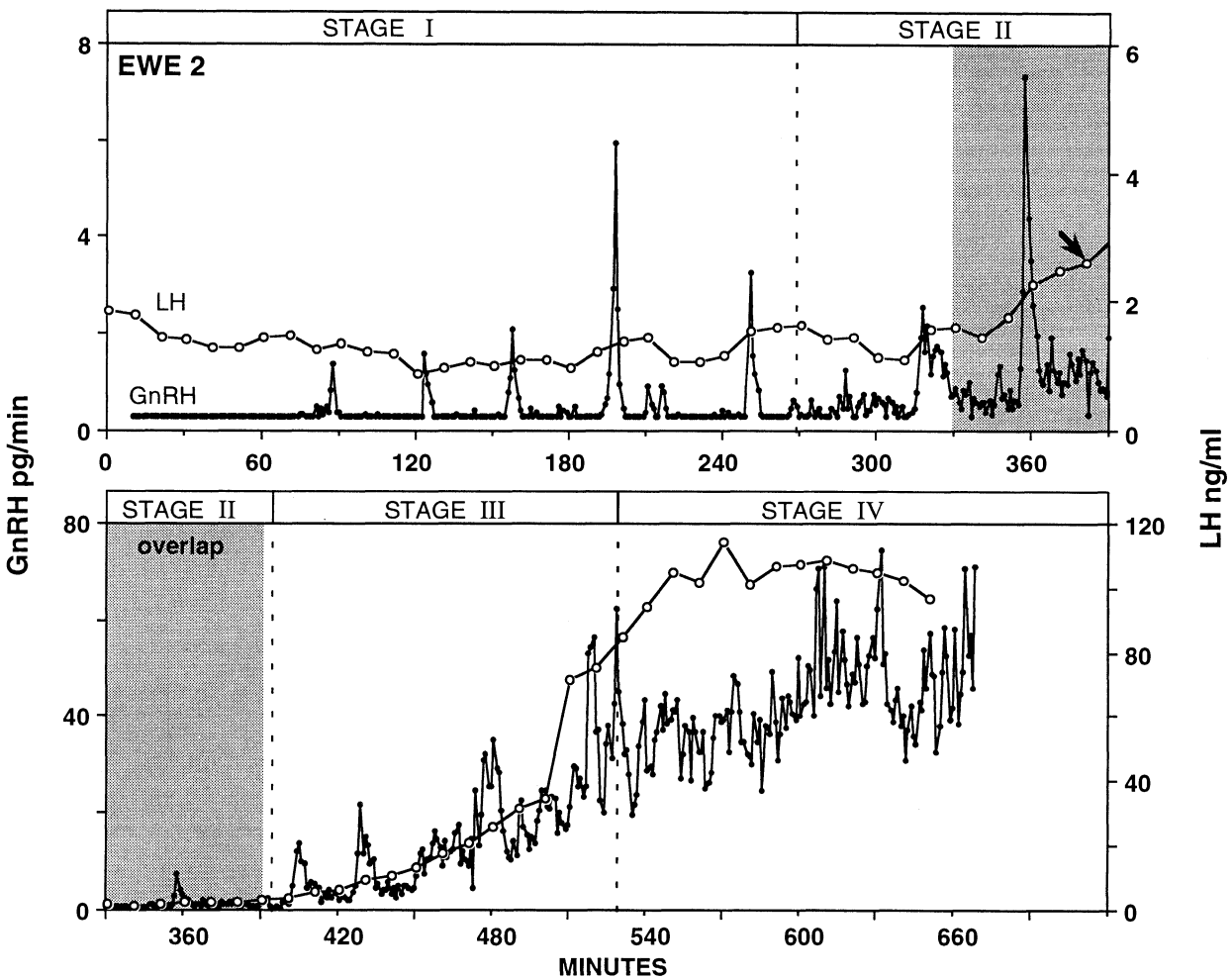


Fig. 6. Representative GnRH patterns (closed circles) in 1-min samples of portal blood and LH (open circles) in 10-min samples of peripheral blood during development of the GnRH surge in the artificial follicular phase model. Top and bottom panels illustrate data for the first and second halves of the 11-h sampling period (note scales for hormonal values differ between panels). Shaded portion represents 60 min of overlapping data, repeated in each panel for continuity. The observation period is subdivided into four stages based on the GnRH pattern (see text for details). Arrow depicts onset of the LH surge.

prolonged, and the amount of GnRH between pulses increased markedly (395–530 min, Stage III). Finally, GnRH became extremely high and variable, and a regular pulsatile pattern was no longer clearly evident (530–660 min, Stage IV). This latter condition persisted for the remainder of the collection and corresponded to the pattern seen around the GnRH peak and declining limb of the surge, as determined in our earlier study (Moenter et al. 1992b).

The changing GnRH secretory dynamics seen during surge development in this study were much the same as those observed in a preliminary experiment in which portal blood was sampled every 30-s from one ewe at the very onset of the surge in the follicular phase model (Fig. 4 in Moenter et al. 1992b). Further, the time course of GnRH in this model resembles that observed when portal blood was sampled at 1-min intervals during development of the spontaneous preovulatory GnRH surge of the natural estrous cycle (Evans et al. 1995b). We thus feel confident that the characteristic change in GnRH secretory dynamics in the artificial follicular phase model is not an artifact of the experimental preparation.

Of interest, the LH surge in most animals was found to begin shortly after GnRH values became consistently detectable between pulses (Evans et al. 1995b). This was before the major increase in either pulsatile or interpulse GnRH secretion (arrow in Fig. 6). Whether or not the development of detectable interpulse GnRH secretion is a critical signal for induction of the LH surge remains to be tested.

POSSIBLE CHANGES IN THE GnRH NEUROSECRETORY SYSTEM DURING THE SURGE

The foregoing observations have prompted us to consider the type(s) of changes in the GnRH neurosecretory system induced by estradiol to provoke the surge (Evans et al. 1995b). In this section, a number of possibilities are suggested. None are fully tested and some are highly speculative.

Quantitative change in GnRH pulse generating system

Perhaps the GnRH surge results from quantitative changes in the activity of those GnRH neurones that produce the coordinated synchronous output of GnRH during a pulse. For example, there could be an extreme increase in frequency, amplitude and/or duration of release such that the GnRH output from one pulse coalesces with that of neighbouring pulses by the time the hormone reaches the portal circulation. Enhanced frequency of GnRH pulses during the surge has, in fact, been suggested from observations during the estradiol-induced surge in ovariectomized ewes (Caraty et al. 1989, Clarke 1993). Although a progressive increase in GnRH pulse frequency does occur in the early to mid-follicular phase of the estrous cycle (Moenter et al. 1991), the moment-to-moment changes in the GnRH pattern at the very onset of the surge in the artificial follicular phase model are not suggestive of a further increase in frequency (e.g., Fig. 6, Stages II, III). Along similar lines, it is of interest to note that multi-unit electrical recordings from hypothalami of monkeys and goats revealed that the volleys of high frequency electrical output, which correlate directly with LH pulses prior to the surge, do not increase in frequency during the LH surge (O'Byrne et al. 1991, Tanaka et al. 1992). In fact, these volleys were found to become less frequent or actually stop. Yet monkeys and goats, like sheep, exhibit surge-type release of GnRH at the time of the LH surge (Xia et al. 1992, Manube et al. 1993, Pau et al. 1993). In addition to frequency, other types of quantitative change in the system that produces GnRH pulses might contribute to generation of the surge. In support of this, we have consistently observed a train of prolonged high amplitude pulses during the rising edge of the GnRH surge in the artificial follicular phase model (Fig. 6 Stage III; see also Moenter et al. 1992b, Evans et al. 1995b).

Desynchronization of GnRH neurones

Since estradiol can produce qualitative changes in the pattern of GnRH secretion (Figs. 4 and 5), it

is possible that the surge results from an altered firing pattern of the same GnRH neurones that previously were coordinated to produce a pulse. For example, GnRH neurones might become progressively desynchronized under the influence of a rise in circulating estradiol. This may lead to altered pulse shape, release between pulses and the eventual disappearance of discrete pulses. In view of the massive amount of GnRH released during the surge as compared to other times of the cycle (Moenter et al. 1991), it is not likely that such desynchronization by itself could account for the surge. If combined with enhanced GnRH synthesis and transport to the median eminence, however, such a qualitative change could be highly important.

Recruitment of surge-specific GnRH neurones

Activation of a separate population of GnRH neurones at the time of the surge could account for the moment-to-moment pattern of GnRH release during the surge, if those neurones were not coordinated to release GnRH in a strictly episodic fashion. Recruitment of a surge-specific group of GnRH neurones is supported by neuroanatomical studies in other species. In rats, for example, the number and distribution of immunocytochemically identifiable GnRH neurones in the hypothalamus changes in association with the preovulatory LH surge (Rubin and King 1994). Further, based on studies using molecular markers of increased cell activity, anatomically separate tonic and surge groups of GnRH cells have been observed in rats and hamsters (Berriman et al. 1992, Porkka-Heiskanen et al. 1994). In sheep, initial efforts to identify a surge-specific population of GnRH neurones, using cfos as a marker of neuronal activation, indicated that GnRH neurones activated during the surge are not confined to a restricted anatomical locus (Moenter et al. 1993). Yet, preliminary evidence in the ewe suggests a restricted cluster of GnRH neurones may be responsible for GnRH secretion during a pulse (Goodman et al. 1994). Additional studies of this type seem warranted in sheep.

Structural alterations of the GnRH neurosecretory system

In other neurosecretory systems, ultrastructural alterations in endocrine neurones and adjacent cells have been described in association with altered states of release. In the oxytocin and vasopressin systems, for example, the number of cell-to-cell interactions changes during periods of enhanced secretion by movement of glial cells and pituicytes (Hatton et al. 1984, Theodosis and Poulain 1992). Evidence has recently been forwarded for similar types of structural reorganization in the GnRH neurosecretory system of rats. For example, GnRH terminals are closely apposed to glial elements in the median eminence, and the distance between GnRH terminals and the portal vasculature can be influenced by gonadal hormones (King and Letourneau 1994, King and Rubin 1994). The likelihood that estradiol interacts directly with glia is suggested by findings that glial elements in the median eminence contain estradiol receptors and that mRNA for the estradiol receptor is present in hypothalamic astrocytes (Langub and Watson 1992, Ma et al. 1994). Moreover, the spatial association of GnRH terminals and tanycyte end-feet in the median eminence has recently been found to change in rats at the time of the preovulatory LH surge (King et al. 1995). In particular, GnRH terminals during the surge are no longer encased by tanycyte end-feet. Instead, they are positioned in closer apposition to portal capillaries. Such morphological changes might alter either the pattern and amount of GnRH release or the efficiency of GnRH delivery into the portal vasculature. Further studies to address this possibility could be highly rewarding.

Change in metabolism and route of delivery of GnRH

Other types of changes, in addition to the morphological alterations just described, could modify the delivery of GnRH once it is released from terminals in the median eminence. For example, the median eminence of sheep contains endopeptidase

enzymes that metabolize GnRH; the activity of these enzymes is reduced by estradiol treatment that elicits an LH surge (Smith et al. 1992). Moreover, enzyme activity was recently found to decrease around the time of the preovulatory LH surge (Law et al. 1995). This reduced enzyme activity, in turn, could increase the amount and/or alter the pattern of GnRH delivered to the pituitary independent of any change in the amount or pattern of hormone released.

Beyond metabolism, the recent finding of increased GnRH in cerebrospinal fluid (CSF) of the third ventricle during the estradiol-induced surge in the ewe provides yet another means by which delivery could change independent of the release process itself (Skinner et al. 1995). Although the route by which GnRH enters the CSF is not known, the hormone is cleared from CSF more slowly than from portal blood (Skinner et al. 1995). Thus, CSF might act as a storage reservoir, with GnRH accumulating in the third ventricle and subsequently diffusing (or being transported) through the median eminence to the portal vasculature. This, in effect, could mask the actual pattern of release at any given time. For example, such diffusion of GnRH during the surge could cause the appearance of a continuous elevation of the hormone in portal blood despite a discrete pulsatile pattern of release.

It should be reiterated that the foregoing explanations for generation of the GnRH surge are highly speculative. They are not mutually exclusive nor do they include all possible ways that estradiol could alter the GnRH neurosecretory system to produce the surge. Not included, for example, are changing activities of the vast array of synaptic inputs to the GnRH neuronal network and the interplay among various neurotransmitters known to regulate GnRH release. The possibilities presented, however, do provide a conceptual framework for stimulating the development of testable hypotheses and future experimentation.

SUMMARY

Much of our knowledge about estradiol feedback actions on GnRH secretion during the preovulatory period has been gained from techniques allowing sampling hypophyseal portal blood of sheep. Beginning with regression of the corpus luteum, marked changes occur in the episodic pattern of secretion. GnRH pulse frequency increases and amplitude decreases in conjunction with the follicular phase rise in estradiol secretion. Coincident with onset of the LH surge is a massive and sustained release of GnRH – the preovulatory GnRH surge. Studies employing a physiological model for the follicular phase of the estrous cycle have revealed that estradiol has profound and complex feedback actions on the pattern of GnRH release during the preovulatory period. These include both quantitative effects on pulses (increasing frequency and decreasing amplitude) and qualitative effects (altering pulse shape and stimulating interpulse secretion), in addition to induction of the preovulatory GnRH surge.

In stimulating the GnRH surge, estradiol causes a highly characteristic change in the minute-to-minute pattern of GnRH in hypophyseal portal blood. Initially, a strictly episodic pattern gives way to one in which GnRH is consistently elevated between pulses. Following enhancement of both the pulsatile and interpulse components, GnRH values become extremely high and variable for the majority of the surge. At this point, a regular and well organized pulse pattern is not readily apparent. The characteristic time course of GnRH during development of the surge provides insight into possible changes in the GnRH neurosecretory system that might lead to generation of the surge. Various types of alterations are consistent with this time course, including quantitative and qualitative changes in the GnRH pulse generating mechanism, recruitment of a surge specific population of GnRH neurones, morphologic alterations in GnRH neurones and adjacent cells, and changes in efficiency or route of delivery of GnRH from its site of release to the portal vasculature.

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