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The preovulatory gonadotrophin-releasing hormone surge: a neuroendocrine signal for ovulation

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Recent studies have demonstrated that an important component of the positive feedback response to oestradiol in mammals is an action within the central nervous system to induce a large surge in the secretion of gonadotrophin-releasing hormone (GnRH). This oestradiol-induced neuroendocrine signal for ovulation has been best characterized in ewes. The GnRH surge is high in amplitude; the amount secreted increases on average more than 40 times above the pre-surge baseline value. The initial increment in GnRH secretion precedes or coincides with the onset of the LH surge. The GnRH surge is of extended duration, lasting far longer than the preovulatory LH surge. A molecular variant of GnRH, which is less active biologically than native GnRH, is co-secreted at the time of the surge, but termination of the LH surge cannot be accounted for by a change in biological activity of the secreted GnRH. Generation of the GnRH surge appears to follow a characteristic progressive change in the pattern of GnRH in portal blood. High concentrations of oestradiol initially stimulate the secretion of GnRH between pulses; this is followed by augmentation of both pulsatile and interpulse GnRH release producing the rising limb of the surge. Finally, recent experiments have indicated that the local application of oestradiol to the ventromedial nucleus of the hypothalamus is sufficient to stimulate the GnRH surge, suggesting a key role for this hypothalamic area in the generation of this neuroendocrine signal for ovulation.

Introduction

The preovulatory surge of gonadotrophic hormones from the anterior pituitary gland is the regulatory pivotal step in the oestrous and menstrual cycle because it induces ovulation. A great deal of effort has been spent to gain an understanding of the factors that regulate the occurrence of the preovulatory gonadotrophin surge. It has long been known that an increase in ovarian oestradiol secretion is required for induction of the preovulatory surges of LH and FSH. In many species including rats (Aiyer *et al.*, 1974), sheep (Reeves *et al.*, 1971) and monkeys (Knobil, 1974), it has been demonstrated that oestradiol increases the pituitary responsiveness to GnRH. However, it has become evident that another important component of the positive feedback response to oestradiol is an action within the central nervous system to induce marked changes in the secretion of GnRH. Historically, this neural component of the positive feedback response has been difficult to characterize owing to the nature of the GnRH neuronal system itself. In this regard, there are only a few thousand GnRH neurones; they are widely dispersed throughout the hypothalamus; and not all of them are in contact with the portal vessels of the median eminence (Silverman *et al.*, 1990). Even in hypophyseal portal blood, where the neurosecretory signal is concentrated, the amount of GnRH is relatively small.

The development of various methods for measurement of GnRH secretion has led to the demonstration that there is an unambiguous and sustained surge of GnRH at the time of the preovulatory LH surge in rats (Sarkar *et al.*, 1976; Ching, 1982), ewes (Domanski *et al.*, 1991; Moenter *et al.*, 1991), monkeys (Xia *et al.*, 1992; Pau *et al.*, 1993) and mares (Irvine and Alexander, 1994). In most of these species, it has also been confirmed that the preovulatory increase in oestradiol plays an essential role in triggering a cascade of neuroendocrine events that culminate in the GnRH surge (Sarkar and Fink, 1979; Clarke, 1988; Caraty *et al.*, 1989; Moenter *et al.*, 1990; Xia *et al.*, 1992). In this report, we summarize these findings and those of subsequent experiments to examine the mechanisms responsible for generation of this neuroendocrine signal for ovulation in ewes.

The Preovulatory GnRH Surge

The first evidence for increased GnRH release into hypophyseal portal blood at the time of the LH surge was obtained in anaesthetized laboratory rats (Sarkar *et al.*, 1976). This observation was facilitated by the availability of a technique for sampling hypophyseal portal blood and the fact that the gonadotrophin surge in this species is precisely timed on the day of pro-oestrus. This result (illustrated in Fig. 1a) was later confirmed by Ching (1982). However, progress in this area in other species was hindered by the lack of reliable techniques for monitoring the pattern of hypothalamic substances, such as GnRH, in hypophyseal portal blood. In 1982, a method for obtaining sequential samples of hypophyseal portal blood from conscious undisturbed sheep was developed by Clarke and Cummins (1982). However, the initial report of the pattern of GnRH secretion at the time of the preovulatory LH surge in ewes did not substantiate the view that an increase in GnRH secretion was an important stimulus for generation of the gonadotrophin surge. Notably, the pattern of GnRH release was found to be highly variable and did not necessarily undergo a noticeable change during the LH surge (Clarke *et al.*, 1987). This finding was more in keeping with the view that the anterior pituitary gland was the primary site for the positive feedback action of oestradiol in sheep, as had been proposed for rhesus monkeys (Knobil *et al.*, 1980).

We were uncomfortable with the inconsistent patterns of GnRH release during the LH surge in ewes, because indirect evidence had been compiled to suggest that, unlike in monkeys, generation of the preovulatory LH surge in ewes requires a large increase in GnRH release (Kaynard *et al.*, 1988). Subsequently, we developed a modified approach for the collection of hypophyseal portal blood that allowed sampling from the same animal for up to 48 consecutive hours (Caraty and Locatelli, 1988). This period was sufficient to span not only the LH surge but also the pre-surge period. We then used this technique to assess the role and importance of changes in GnRH secretion to the generation of the preovulatory sequence of events in ewes.

In our initial studies, portal and jugular blood were collected at intervals of 10 min for 24–48 h across the period leading up to and during the expected time of the preovulatory LH surge in intact cyclic ewes (Moenter *et al.*, 1991). Owing to the difficulty in accurately predicting the time of onset of the LH surge in intact animals, it was necessary to use a large number of sheep. This experiment, was therefore conducted over two years in our laboratories in France and Michigan, and included two breeds of sheep, Ile de France and Suffolk. In each of 11 ewes in which portal blood was sampled during all or part of the LH surge, we obtained evidence for an unambiguous increase of GnRH release. An example of the dynamics of GnRH and LH release over 48 continuous hours leading to ovulation in one ewe is shown (Fig. 1b). Several points are of particular interest. As the follicular phase progressed and oestradiol secretion increased, there was initially a reduction in GnRH pulse amplitude and often an increase in pulse frequency. A robust GnRH surge then occurred coincident with the onset of the preovulatory LH surge. During this surge, a strictly pulsatile pattern of secretion was not clearly evident as GnRH values remained continuously high. Our finding and those from a study using push–pull perfusion of the median eminence (Domanski *et al.*, 1991) provide definitive evidence for the existence of a preovulatory GnRH surge in ewes.

Finally, recent reports in monkeys (Xia *et al.*, 1992, Pau *et al.*, 1993) and mares (Irvine and Alexander, 1994) involving various methods have provided evidence for the existence of an unambiguous GnRH surge at the time of the LH surge in these species. A representative example of the pattern of GnRH and

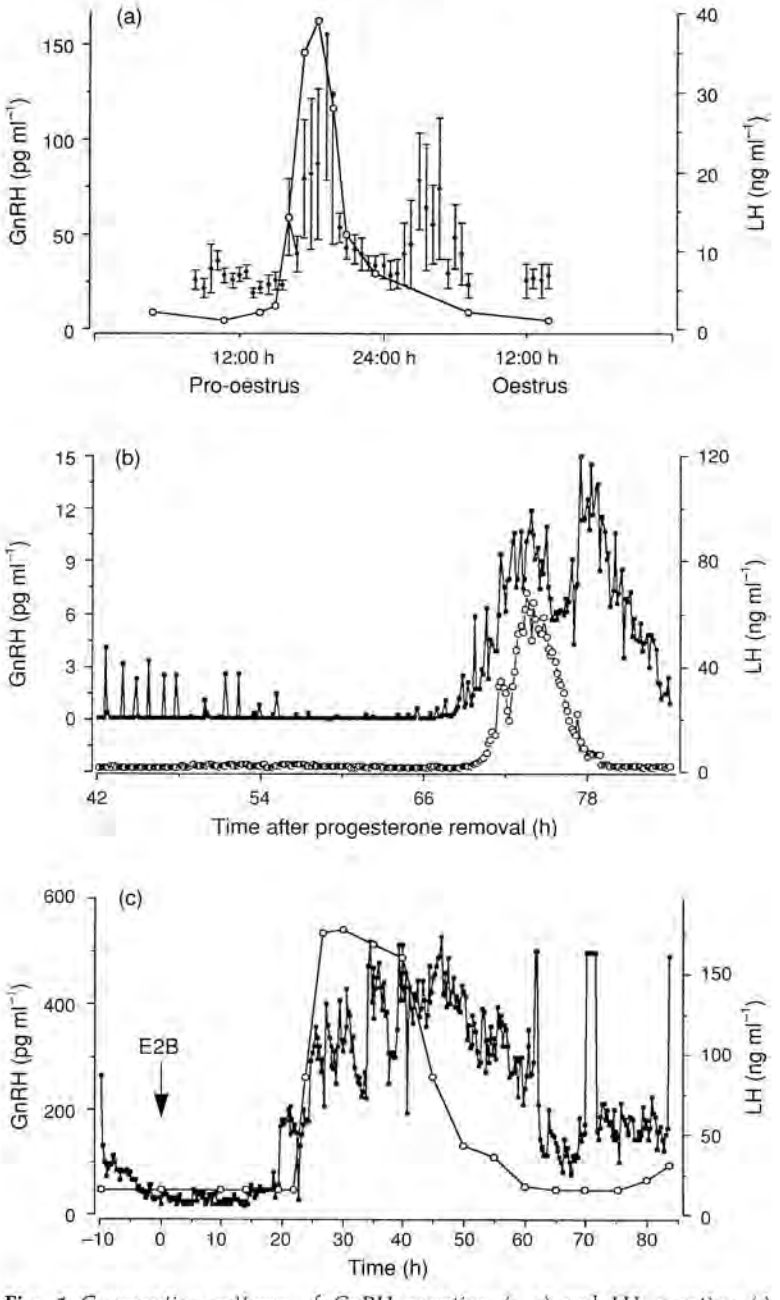

Fig. 1. Comparative patterns of GnRH secretion (▪, •) and LH secretion (○) (a) during the day of pro-oestrus and oestrus in rats (redrawn from Sarkar *et al.*, 1976), (b) during the late follicular phase of the oestrous cycle in a representative ewe (redrawn from Moenter *et al.*, 1991) and (c) after administration of oestradiol benzoate (E2B) in intact monkeys (redrawn from Xia *et al.*, 1992).

LH released in response to oestradiol benzoate administration in an intact monkey is shown (Fig. 1c). Certain characteristics of the GnRH surges observed in these three species are similar. In each case, the amplitude of the GnRH surge is large and the surge lasted far longer than the preovulatory LH surge.

This latter observation indicates that the end of the LH surge is not due to lack of GnRH, and this point is considered in greater detail later. As the GnRH surge appears to be a common neuroendocrine signal for ovulation in many species, it is important to consider the mechanisms that lead to its generation.

Role of Oestradiol in the Induction of the GnRH Surge

In ovariectomized ewes, it has been widely shown that oestradiol inhibits LH secretion before inducing a large release of LH that is similar to the spontaneous preovulatory LH surge (Pelletier and Signoret, 1969). However, the first studies to describe the effects of large doses of oestradiol on GnRH secretion did not provide definitive results, as the patterns of GnRH secretion obtained were not consistent among animals (Clarke and Cummins, 1985; Schillo *et al.*, 1985). We therefore used the technique developed for the collection of hypophyseal portal blood to study the pattern of GnRH secretion following intravenous injection of a bolus of oestrogen to ovariectomized ewes during the breeding season (Caraty *et al.*, 1989). Oestradiol induced the expected biphasic pattern of LH release in the peripheral circulation, an initial decrease (negative feedback) followed by an increase (positive feedback). Of greater interest, a similar biphasic pattern was also observed for GnRH in the hypophyseal portal circulation. During the period of negative feedback of LH secretion, a clear but less marked decrease was observed in both GnRH pulse frequency and amplitude. During the positive feedback phase, a large and unambiguous surge of GnRH occurred coincident with the LH surge. It was also observed that, much as during the preovulatory GnRH surge, the pattern of GnRH release changed such that GnRH was continuously high and discrete pulses were no longer evident.

These results, in conjunction with earlier observations that administration of a bolus of oestradiol to intact anoestrous ewes stimulated a clear increase in GnRH secretion at the time of the LH surge (Clarke, 1988), provide the first direct evidence that an abrupt increase in GnRH secretion is a key component of the positive feedback effects of oestradiol on LH secretion in ewes. However, as the circulating concentration of oestradiol achieved in our first experiment was calculated to be well above the physiological range, we next studied the pattern of GnRH secretion at the time of the LH surge in a previously developed physiological experimental model (Goodman, 1988). In this model, the steroidal milieu of the follicular phase of the oestrous cycle is simulated to produce an artificial follicular phase. Separate experiments were conducted during both the breeding and anoestrous seasons.

A representative pattern of GnRH secretion at the time of an LH surge induced in the artificial follicular phase model during the breeding season is illustrated in Fig. 2 (Moenter *et al.*, 1990). Before the LH surge, the concentration of GnRH in portal blood was very low owing to the negative feedback effects of the peak follicular phase concentrations of oestradiol. In all animals, this was followed by an unambiguous surge of GnRH secretion. The absolute requirement of oestradiol for induction of this surge was demonstrated by the absence of an increase in GnRH secretion in control animals that did not receive oestradiol. The induced GnRH surge was essentially indistinguishable from the spontaneous preovulatory GnRH surge. It invariably began coincident with the LH surge but continued well beyond the LH surge, again providing evidence that the LH surge is terminated for reasons other than a lack of GnRH. The induced surge was of high amplitude; peak GnRH concentrations typically exceeded the pre-surge baseline value by more than 100 times. Finally, we found that the time course and magnitude of the GnRH surges induced in the artificial follicular phase model did not differ between the breeding and anoestrous seasons. The demonstration of consistent unambiguous surges of GnRH secretion in these studies using a physiological model of the follicular phase provides compelling evidence that the preovulatory rise of oestradiol secretion causes an abrupt and robust activation of the GnRH neurosecretory system, before ovulation, in ewes.

Nature of the GnRH Secreted During the Surge

As described above, we have consistently observed that the spontaneous and oestradiol-induced GnRH surges continue for many hours beyond the LH surge. One implication of this observation is that the LH surge terminates for reasons other than a lack of GnRH, for example pituitary depletion or

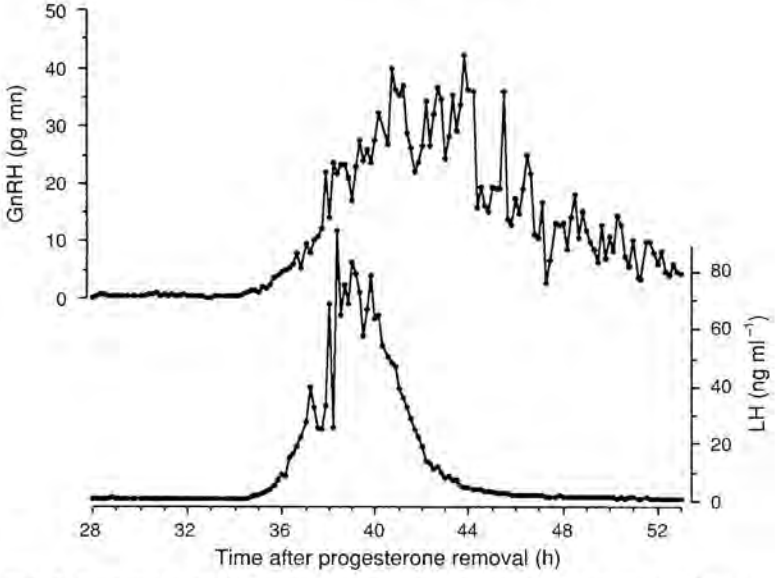

Fig. 2. Pattern of GnRH in hypophyseal portal blood and LH in jugular blood in a representative ewe in the follicular phase model. The oestradiol rise was initiated 16 h after removal of progesterone. Redrawn from Moenter *et al.* (1990).

desensitization. However, there is another possibility. Recent studies have demonstrated the presence of at least one other molecular form of GnRH, hydroxyproline-9-GnRH (Hyp-GnRH), in the hypothalami of a variety of vertebrates including rats, mice, frogs, humans and sheep (Gautron *et al.*, 1991). Moreover, Hyp-GnRH is less potent than native GnRH in stimulating the release of LH and FSH from the pituitary (Gautron *et al.*, 1992). Thus, termination of the LH surge might be due to a change in the form of secreted GnRH from the native decapeptide to a less biologically active molecule such as Hyp-GnRH. It should be noted, with regard to this explanation, that GnRH surges sometimes exhibit a biphasic profile (Fig. 1b), suggesting possible successive release of two forms of GnRH. In an attempt to resolve this issue and to study the nature of GnRH secreted during the surge, we conducted the three experiments summarized below.

In the first experiment, GnRH immunoreactivity was determined in hypophyseal portal blood during an oestradiol-induced GnRH surge using two different antibodies having respective specificity toward the amino- and carboxyl-terminal portions of the GnRH molecule (Caraty *et al.*, 1993). Binding to the first antibody (BDS antibody) was found to require the first seven amino acids of the GnRH molecule and, therefore, the antibody recognizes both native GnRH and molecules having modifications in the carboxyl-terminal portion of the molecule (for example Hyp-GnRH). However, binding to the second antibody (CRR antibody) requires the last five amino acids of the GnRH molecule. This antibody, therefore, does not bind carboxy-terminal modifications of the native GnRH decapeptide (that is, does not bind Hyp-GnRH). These antibodies were used to study the patterns of immunoreactivity in samples of hypophyseal portal blood collected during oestradiol-induced GnRH surges. Patterns of GnRH immunoreactivity measured with each of these antibodies from the same samples of hypophyseal portal blood of one representative animal are illustrated (Fig. 3). For all animals ($n = 8$), there was no significant difference in either the pattern, quantity, time of onset, or duration of GnRH secreted during the surge. Owing to the specificity of the antibodies, this experiment provides evidence that GnRH released during the surge is mainly of one form, the native GnRH.

In the second experiment, we tested whether the GnRH molecules released during the latter part of the GnRH surge are biologically active. Our approach was to block the ability of the pituitary to respond to GnRH for several hours at the beginning of the surge by administration of a GnRH antagonist. If the GnRH released during the latter part of the surge is biologically active, then an

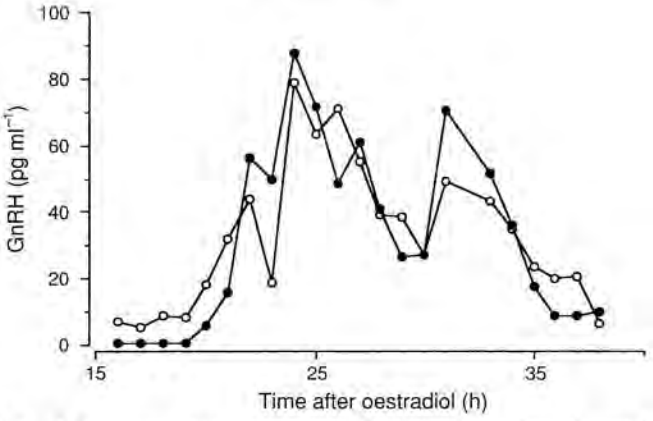

Fig. 3. Immunoreactive patterns of GnRH in hypophyseal portal blood measured with an antibody specific for the amino-terminal portion (•) or the carboxy-terminal portion (○) of the GnRH molecule throughout a GnRH surge induced in the follicular phase model. Redrawn from Caraty *et al.* (1992).

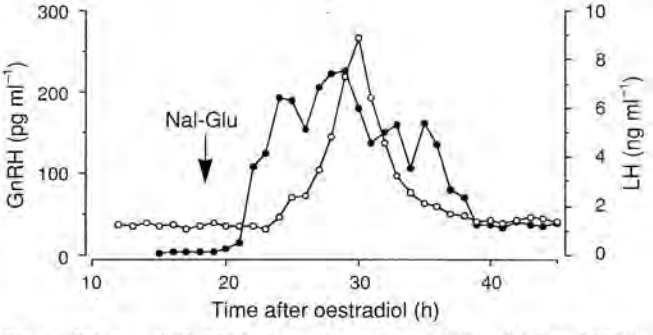

Fig. 4. Pattern of GnRH in hypophyseal portal blood (•) and LH in jugular blood (○) throughout an oestradiol-induced GnRH surge of a representative ewe. Time of administration of the GnRH antagonist (Nal-Glu) is indicated by the arrow. Redrawn from Caraty *et al.* (1992).

increase in LH would be expected to occur once the effect of the antagonist had worn off (that is, during the latter part of the GnRH surge). Ewes set up in the follicular phase model were treated with the antagonist Nal-Glu, 2–3 h before the expected onset of the LH and GnRH surges. (Nal-Glu binds competitively to the GnRH receptor.) The dose of Nal-Glu used was determined in pilot experiments to block pituitary responsiveness to GnRH for 6–10 h. As expected with the follicular phase model, a surge of GnRH began about 20 h after administration of oestradiol but an LH surge did not begin at that time, owing to the action of Nal-Glu. Of interest, a marked increase in LH did begin about 5 h later (Fig. 4). (The magnitude of the LH increase was less than that of LH surges shown in Figs 1 and 2, but this is due in part to different assay standards used in France and Michigan.) This LH response during the latter part of the GnRH surge, in conjunction with recent findings that the GnRH present in portal blood obtained late in the surge can stimulate gonadotrophin secretion *in vitro* (Padmanabhan *et al.*, 1994) demonstrates that at least some of the GnRH molecules released at the end of the surge are biologically active. Together with the results of the first experiment, this indicates that a change in the form of the GnRH from the native peptide to a less active molecule is not the reason for termination of the LH surge.

To determine whether any other GnRH forms are secreted in hypophyseal portal blood during the surge, we conducted a third experiment (Delaleu *et al.*, 1993). Pools of hypophyseal portal blood corresponding to the first and second halves of the oestradiol-induced GnRH surges were submitted to purification on a reverse phase HPLC column as described by Gautron *et al.* (1991). The elution fractions were then assayed using the two antibodies having specificity against the amino- and carboxyl-portions of the GnRH molecule described above. During both the first and second halves of the surge, most of the GnRH molecules behaved immunologically and biochemically as the native decapeptide. A small proportion of a second form of GnRH was also observed; it had an elution pattern identical to that of Hyp-GnRH and was not recognized by the antibody specific to the carboxyl-terminal portion of the GnRH molecule. However this modified form of the GnRH molecule represented only $16.7 \pm 3.3\%$ ($n = 5$) of the total immunoreactivity and did not change between the first and second halves of the GnRH surge.

Collectively, the results of these three experiments indicate that one major form of GnRH is secreted during the entire surge and that this form is the biologically active decapeptide. Thus, termination of the LH surge before the end of the GnRH surge must be due to reasons other than a lack of biologically active GnRH. These could include exhaustion of the releasable pool of LH in the pituitary (Crowder and Nett, 1984) or pituitary desensitization (Nett *et al.*, 1981).

Mode of Activation of the GnRH Neurosecretory System

The magnitude of the GnRH surge raises intriguing questions related to both the GnRH neurosecretory process and the mode of action of oestradiol. For example, does oestradiol induce the GnRH surge by accelerating or heightening the episodic pattern of release? Alternatively, does oestradiol elicit the surge by causing a switch from a pattern of secretion that is strictly pulsatile to a pattern that produces a continuous release of GnRH in portal blood.

Studies with ewes (Clarke and Cummins, 1985; Caraty *et al.*, 1989) led to the conclusion that, during the oestradiol-induced LH surge, there is an increase in GnRH pulse frequency as well as an increase in the basal concentration of GnRH. However, the relatively long interval between blood samples (5–10 min) in those studies precluded accurate characterization of the dynamics of GnRH release at the time of the surge. The moment to moment pattern of secretion during the GnRH surge was later re-examined by Moenter *et al.* (1992a) using more frequent sampling. By taking portal samples every 30 s, it was first demonstrated that in ovariectomized ewes, GnRH secretion is strictly pulsatile (Moenter *et al.*, 1992b). In this situation, the dynamics of a GnRH pulse resembled a square wave, consisting of 5–8 min of high secretion followed by between pulse periods when there is little or no detectable GnRH release. However, during the surge, the pattern was markedly different. Despite considerable fluctuations between contiguous points suggesting a variable rate of release, GnRH values remained continuously high with no consistent evidence for a period without release. Although a component of episodic release cannot be totally eliminated on the basis of that study, it was suggested that an element of continuous GnRH release occurs during the surge. This led to the hypothesis that oestradiol may induce the GnRH surge by causing a switch from a strictly pulsatile pattern of secretion to a pattern that leads to an uninterrupted discharge of decapeptide.

As an initial test of this hypothesis, very frequent samples of pituitary portal blood were collected from ovariectomized ewes exposed to different oestradiol concentrations, to determine whether oestradiol induces qualitative changes in the pattern of GnRH secretion (Evans *et al.*, 1993a). It was observed that, although GnRH patterns were strictly pulsatile in ovariectomized ewes, treatment with increasing concentrations of oestradiol stimulated the release of significant amounts of GnRH between pulses. Thus, we postulated that the preovulatory rise in oestradiol enhances the release of GnRH between pulses, leading to continuously high secretion during the preovulatory GnRH surge. In another experiment, the moment to moment changes in GnRH secretion during the onset of the GnRH surge were examined, to trace the development of the interpulse GnRH release and thereby gain additional insight into its contribution to the generation of the surge (Evans *et al.*, 1993b). The follicular phase model was set up in seven ewes and samples of hypophyseal portal blood collected at intervals of 1 min

for 11 h, thus encompassing the onset of the GnRH surge. Preliminary analysis of our findings revealed that a consistent qualitative change in the mode of GnRH secretion occurs at the start of the surge. Initially, GnRH secretion changed from being strictly pulsatile (that is, no detectable GnRH between pulses) to pulsatile in combination with significant interpulse secretion. This was followed by augmentation of both pulsatile and inter-pulse components, before a prolonged period during which GnRH secretion, although greatly increased, was not organized in a pattern of obvious pulses. Onset of the LH surges in this experiment coincided with the emergence of detectable release of GnRH between pulses. In a similar type of study in which portal blood was sampled at intervals of 2 min, Clarke (1993) observed a highly variable pattern of GnRH secretion among ewes at the start of the surge. This is in marked contrast to the consistent pattern among ewes in our studies described above. This discrepancy may be due to procedural differences in collecting portal blood or to the different animal models used in our respective laboratories (long-term ovariectomized ewes given a bolus injection of oestradiol benzoate in Clarke's studies as opposed to the artificial follicular phase model in our experiments).

Although the reasons for the differences between our findings and those of Clarke (1993) are not known, the results obtained in our laboratory support the hypothesis that oestradiol induces the GnRH surge by changing the organization of the firing pattern among GnRH neurones from a pattern that is synchronous and causes a strictly pulsatile release to an uninterrupted discharge of GnRH into portal blood. Such changes may reflect one or more of several mechanisms including: progressive desynchronization of GnRH neurones that previously fired in unison; extreme acceleration of frequency of episodic release such that the interval between bursts is less than can be resolved by the sampling system; recruitment of a surge-specific population of GnRH neurones that fire in a non-synchronous fashion. However, the magnitude of the GnRH surge relative to that of the antecedent pulses makes it unlikely that the surge is due simply to desynchronization. In rats, the presence of a surge-specific population of neurones is supported by data showing recruitment of GnRH neurones expressing c-Fos activity during the ascending phase of the pro-oestrous surge (Lee *et al.*, 1992) and by a regional pattern of periovulatory GnRH gene expression at the time of the surge (Porkka-Heiskanen *et al.*, 1993). In ewes, c-Fos expression also increases during the oestradiol-induced GnRH surge (Moenter *et al.*, 1993), but it is without a specific regional distribution. Among the other possibilities, it should be noted that the episodic pattern of hypothalamic electrical activity associated with pulsatile LH secretion is not observed during the LH surge in monkeys and rats (O'Byrne *et al.*, 1991; Nishihara *et al.*, 1994) and decreases in goats (Tanaka *et al.*, 1992). These data could be interpreted in two ways. Either the positive feedback effects of oestradiol on GnRH secretion are exerted through a neuronal mechanism that is intrinsically different from the system responsible for pulsatile release of GnRH, or a desynchronization of the GnRH neurones during the surge precludes detection of an episodic electrical signal. Clearly, further investigation is necessary to determine the mechanism(s) by which the increment of oestradiol activates the GnRH neuronal system.

Site of Oestradiol in Induction of the GnRH Surge in Ewes

Given the crucial role of oestradiol in inducing the GnRH surge, another important question emerges. Where in the central nervous system does oestradiol act to stimulate GnRH secretion? Although the answer is not known for ewes, it is pertinent to note that, as in rats (Shivers *et al.*, 1983), GnRH neurones in ewes appear to contain few, if any, receptors for oestradiol (Lehman and Karsch, 1993). Therefore, the effect of oestradiol on the activity of these neurones must be mediated by an oestrogen-sensitive afferent neuronal system. In rats, the rostral medial preoptic area (MPOA) is required for oestrogen-induced LH surges (Petersen *et al.*, 1989). In ewes, placement of oestradiol implants into the ventromedial nucleus of the hypothalamus, but not in the preoptic area, was effective in eliciting both oestrous behaviour and LH release (Blache *et al.*, 1991). On the basis of the latter finding, we recently conducted experiments to determine where in the hypothalamus oestradiol acts to elicit the GnRH surge in ewes.

Sheep were ovariectomized and immediately treated with oestradiol and progesterone to simulate naturally occurring changes in steroid concentrations, thus creating repeated artificial oestrous cycles. In some cycles (positive controls), the oestradiol stimulus for the GnRH surge was provided by

subcutaneous implants. In other cycles, oestradiol was given using implants placed in the ventromedial nucleus of the hypothalamus. Negative controls received cholesterol implants in the hypothalamus. Hypophyseal portal blood was sampled once an hour to monitor the secretion of GnRH over about 30 h. Thus far, we have obtained data on five ewes in which oestradiol implanted into the ventromedial nucleus induced a GnRH surge and five others in which hypothalamic implants were ineffective in this regard. We are currently evaluating whether there is a relationship between the effectiveness of the brain implants in inducing the surge and the density of oestradiol receptors in close proximity to the implantation sites. For those ewes that exhibited a GnRH surge, the pattern of the GnRH response induced by the brain oestradiol implants was similar to that of the positive control ewes treated peripherally with oestradiol. However, the amplitude of the GnRH surges induced by the brain implants appeared to be slightly less than that in the positive controls, suggesting that not all of the stimulatory inputs to the GnRH neuronal network had been activated. None of the negative control animals treated with hypothalamic implants of cholesterol responded with a surge.

These findings provide strong evidence that oestradiol acts, at least in part, in or around the ventromedial nucleus of the hypothalamus to induce the GnRH surge. Moreover, since GnRH surges were induced in only some ewes with implants in this location, the site for the positive feedback action of oestradiol in ewes would appear to be rather confined. Taken together with the fact that oestradiol probably does not diffuse more than one millimetre from the tip of the implant (Blache *et al.*, 1991), a possible action of oestradiol in more anterior regions of the hypothalamus seems unlikely. This is in contrast to the positive feedback effect of oestrogen in rats which seems to be restricted to the rostral medial preoptic area (Petersen *et al.*, 1989). Our results, however, are consistent with earlier evidence that LH surges can be induced in ewes by oestradiol implants placed in the ventromedial region but not in the preoptic area (Blache *et al.*, 1991), and they suggest that anterior structures do not constitute a main site for the positive feedback action of oestradiol in sheep. In this regard, it is notable that 34% of the neurones of the medial basal hypothalamus of ewes were found to exhibit increased electrical activity following i.v. injection of oestradiol (Thiery, 1975). Furthermore, a large population of immunoreactive cells containing the oestrogen receptor has been identified in the ventrolateral–ventromedial hypothalamus of ewes (Lehman *et al.*, 1993; Blache *et al.*, 1994). Finally, a change in the density of immunoreactive cells containing oestrogen receptors during the course of the oestrous cycle of ewes has been found in the ventromedial nucleus, but not in other regions of the hypothalamus (Blache *et al.*, 1994). Although our findings do not exclude an action of oestradiol elsewhere in the brain, they provide evidence that the ventromedial nucleus is an important site for the positive feedback effect of oestrogen on GnRH secretion in ewes. Additional studies are now required to describe the anatomical and neurochemical links between neurones in the mediobasal hypothalamus bearing oestradiol receptors and the GnRH neuronal system.

Conclusion

A large and sustained preovulatory GnRH surge is an important neuroendocrine signal delivered by the hypothalamus in response to increased ovarian secretion of oestradiol during the follicular phase of the cycle. This neuroendocrine signal, which is massive and sustained, appears to be a common component of the gonadotrophin surge mechanism for many species. In ewes, this surge probably results from an action of oestradiol within, or near, the ventromedial nucleus of the hypothalamus. By acting at this site, oestradiol appears to cause a switch in the mode of GnRH secretion from a strictly episodic to a sustained increase in GnRH in portal blood. Many important questions must still be answered before we fully understand the steps for generation of this neuronal event, which is crucial to ovulation and thus successful reproduction.

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