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ABSTRACT
The preovulatory LH surge in the sheep is accompanied by a massive and sustained surge of GnRH. The objective of this study was to examine the duration of the endogenous GnRH signal required to induce and maintain a LH surge of full amplitude and duration. For this purpose, we assessed the effect of a competitive GnRH receptor antagonist (Nal-Glu), administered at various times relative to the LH surge, on the development and progression of the surge pattern of LH release. All studies were conducted in a physiological model for the follicular phase of the estrous cycle (artificial follicular phase). In this model, as during the natural follicular phase, the onset of the LH surge is coincident with the initiation of a massive and sustained rise in GnRH secretion. The experimental approach was validated in a preliminary study by determination that the GnRH antagonist could block the LH surge without compromising GnRH release, as measured in pituitary portal blood. In the main experiment, 25 ewes were run through five successive artificial follicular phases, during which the antagonist was not given (control) or was administered before the LH surge, during its ascending limb, or during the descending limb. Treatment with antagonist before the expected time of the surge prevented the LH surge. Treatment during the ascending limb of the LH surge interrupted the rise in LH and caused a prompt cessation of the surge. Treatment during the descending limb of the LH surge resulted in a faster decline in circulating LH concentrations than in control cycles and caused premature termination of the LH surge. Our results are consistent with the conclusion that development and progression of the preovulatory LH surge in sheep depend upon GnRH stimulation throughout its entire time course. (Endocrinology 137: 4730–4737, 1996)

The preovulatory gonadotropin surge in spontaneous ovulators is induced by an increase in circulating estradiol, which stimulates LH and FSH secretion by enhancing both GnRH release and pituitary responsiveness to the releasing hormone (1). The preovulatory pattern of GnRH secretion has been well characterized in sheep due to the availability of a method for sampling hypophyseal portal blood from conscious, normally behaving animals (2–5). In this species, both the spontaneous and estradiol-induced gonadotropin surges have been shown to be accompanied by a large and sustained increase in GnRH release. This GnRH surge begins coincident with the LH and FSH increase but continues for many hours after gonadotropin secretion returns to baseline (3, 4, 6–8). A similar GnRH surge, which extends beyond the gonadotropin surge, has been described for the rhesus monkey (9), rat (10, 11), and mare (12).

The extended duration of the GnRH discharge raises an intriguing question. How much of the sustained GnRH surge is required to induce and maintain the preovulatory LH surge? Experiments employing several different approaches have led to the conclusion that in sheep, at least some of the GnRH surge is necessary (1, 13–18). For example, when estradiol was administered to sheep in which GnRH release had been blocked, it was necessary to provide a bolus of GnRH over and above an ongoing episodic delivery of the releasing hormone for a preovulatory-like surge of LH to be induced (1). In none of these early studies, however, was the essential component of the endogenous GnRH surge determined.

The aim of this study was to determine how much of the endogenous GnRH surge is required to induce and maintain a LH surge of full amplitude and duration. Our specific focus was on the duration of the required GnRH signal. The approach was to monitor the effect of a competitive GnRH receptor antagonist administered at various times relative to the LH surge on the development and progression of the surge pattern of LH release.
Materials and Methods

General

All experiments were conducted using adult Suffolk ewes maintained under natural husbandry conditions at the Sheep Research Facility in Ann Arbor, MI (42° 18' N). The GnRH antagonist used in the present studies was the competitive receptor blocker, Nal-Glu (AC-n2-Nal', D4-C1-Phe', N3-Phe', Gln-Phe', Ile-Phe', Arg-Phe', P-phenylmethylbenzoyl-L-aminobutyric acid', p-Ala-Glu-Nal). Nal-Glu was synthesized at The Salk Institute and made available by the Contraceptive Development Branch, Center for Population Research, NICHD. All studies were performed using a well characterized physiological model for the follicular phase of the estrous cycle (artificial follicular phase model described below) (4, 6, 19, 20). In this model, as during the natural follicular phase, a massive and sustained GnRH surge develops coincident with the LH surge (3, 6). The timing of the surge in the model, however, is more predictable. This facilitated administration of the antagonist at desired times during the course of the surge. In the main experiment, the LH surge was used as an index of the time course of the associated increase in GnRH. This was deemed appropriate due to the coincident increases in GnRH and LH we have invariably observed at the start of the surge, either in the model or during the natural follicular phase (3, 4, 6–8). All experimental procedures were approved by the committee for the use and care of animals at the University of Michigan.

Exp 1: validation of approach

The objective of this experiment was to validate the fundamental assumption of the approach that Nal-Glu can block the LH surge without blocking the GnRH surge in the artificial follicular phase model. This study was begun in the midanestrous season (June); previous studies have demonstrated that comparable GnRH/LH surges are induced in the model during both the breeding and anestrous seasons (6). Five ewes were run through a natural follicular phase separated by artificial luteal phases. These endocrine conditions were produced as follows. The ewes were ovariectomized and treated immediately thereafter with 1-cm SC SILASTIC brand (Dow Corning, Midland, MI) capsule containing estradiol (21) and two 4 × 7-cm SC SILASTIC brand packets containing progesterone (22). These implants maintain serum concentrations of estradiol and progesterone equivalent to those observed during the midluteal phase of the estrous cycle (~1 pg/ml and 3 ng/ml, respectively) (19, 23). After 2 weeks, the progesterone implants were removed to stimulate luteolysis, and 16 h later, four 3-cm SILASTIC capsules containing estradiol were inserted SC to reproduce the preovulatory rise in serum estradiol concentrations to about 8 pg/ml (19). To stabilize the rate of release of estradiol from the SILASTIC brand capsules and thus prevent acute pharmacological alterations in estradiol concentrations, all implants were soaked in water for 24 h before insertion. Restoration of the follicular phase changes in circulating steroid concentrations in this manner reliably induces preovulatory-like surges of GnRH and LH (6). The estradiol implants were removed after 48 h, and the progesterone packets were reimplanted to produce a second artificial luteal phase. This was followed by a second artificial follicular phase approximately 16 days later, the duration of the estrous cycle of the ewes. The cycle of implant changes was then repeated to generate the third artificial luteal and follicular phases. During the first artificial follicular phase (cycle 1), hourly samples of peripheral blood were collected to characterize the LH surge in each animal. During the second artificial follicular phase, animals were treated with Nal-Glu (45 μg/kg, iv) twice, at 16-h intervals, beginning 5 h before the expected time of the LH surge, as judged from surge onset in the first artificial follicular phase. Blood samples were collected at hourly intervals by jugular venipuncture to confirm that this treatment was sufficient to block the LH surge. In a pilot experiment (data not shown), we determined that a single injection of this dose of Nal-Glu suppressed the LH secretion in ovximized ewes for approximately 15 h and, therefore, that our treatment protocol should create a period of suppression sufficient to span the estradiol-induced LH surge in the artificial follicular phase model (LH surge lasts 8–12 h; GnRH surge lasts 18–28 h). Animals were then run through an intervening artificial luteal phase, during which they were prepared surgically for the collection of pituitary portal blood as previously described (3, 6, 20). The influence of Nal-Glu on the secretion of GnRH as well as LH was determined in the third artificial follicular phase (antagonist treatment as in cycle 2). Integrated hourly samples of pituitary portal and jugular blood for measurement of GnRH and LH, respectively, were collected for 21 h at the expected time of the GnRH and LH surges using a modification (20) of the technique of Cortay and Locatelli (5). This procedure permits collection of samples from conscious normally behaving ewes. Pituitary portal blood was collected into 3 × 10^{-3} M ice-cold bacitracin (Sigma) to inhibit endopeptidase activity.

Exp 2: duration of GnRH stimulus required for LH surge

Our objective was to determine how much of the GnRH surge, in terms of duration, is required to induce and maintain a LH surge of full amplitude and duration. The experiment was conducted during the breeding season. Twenty-five ewes were ovariectomized during the midluteal phase (6–11 days after estrus) and immediately treated with steroid implants that maintained luteal phase concentrations of progesterone and estradiol, as in Exp 1. Ewes were studied over five consecutive artificial follicular phases separated by artificial luteal phases, as described in Exp 1. Each artificial estrous cycle was approximately 16 days in length. During the artificial follicular phases of each cycle, samples of jugular blood for LH assay were obtained at hourly intervals beginning 6 h after implanting estradiol and continuing for 36 h. Treatments during the five artificial estrous cycles were as follows.

Cycle 1 (control cycle). Animals were not treated with the antagonist; the onset and time course of the LH surge were characterized in each of the 25 ewes.

Cycle 2 (experimental cycle). Twenty of the 25 ewes were treated with 2 injections of Nal-Glu (65 μg/kg, iv, 10 h apart) at various times relative to the anticipated LH surge. Based on the time of the LH surge in cycle 1, the first of the 2 Nal-Glu injections was given either before the surge or during the expected time of its ascent or descent. The remaining 5 animals were designated untreated controls in this and all subsequent cycles and were not treated with antagonist.

Cycle 3 (control cycle). This was a validation cycle in which we determined whether treatment with the antagonist during cycle 2 affected subsequent surge generation. During this cycle, none of the 25 animals received antagonist treatment, and the occurrence, timing, and characteristics of the LH surge were compared to those in cycle 1.

Cycles 4 and 5 (experimental cycles). The final two cycles again evaluated the influence of antagonist administered at various times relative to the LH surge (n = 20 and 18 in cycles 4 and 5, respectively). Nal-Glu was again administered iv at a dose of 45 μg/kg, but treatment was repeated at 8-h rather than 10-h intervals. (This change was introduced because we observed slight increases in LH in some animals approximately 9 h after treatment with the first Nal-Glu injection in cycle 2, suggesting escape from antagonist action.)

Assays

GnRH concentrations were measured in a previously described RIA (5, 6). A 750-μl aliquot of sample (~600 μl portal plasma and the remainder bacitracin) was extracted in methanol, and duplicate aliquots of the reconstituted extract (equivalent to ~240 μl portal plasma) were assayed. All samples were measured in a single assay. Intray assay variation, as determined by the median variance ratio of assay replicates (24), averaged 0.039, and assay sensitivity was 0.097 pg/tube. LH was measured in duplicate aliquots of plasma (25–200 μl) using a modification (25) of a previously described RIA (26, 27). Values are expressed in terms of NIH LH-S12. Mean inter- and intraassay coefficients of variation (31 assays) were 8.28% and 11.4%; assay sensitivity averaged 0.61 ng/m.

Data analysis

The presurge baseline (BAS) was defined as the mean LH concentration 6–10 h after the addition of estradiol implants, when values are low. (LH surge onset in this model occurs, on the average, 18.5 h after the addition of estradiol implants.) The start of the LH surge was defined as the time at which LH exceeded BAS + (2 × sd) and remained at or above this level for at least 3 h. The end of the LH surge was more...
difficult to identify due to increased variability in the LH pattern at the end of the surge relative to that before its start. The end of the LH surge was defined as either the time that LH concentrations fell below 2 × BAS for the time that an increase was seen between consecutive samples (if this occurred after a prolonged and consistent decrease to concentrations that approached 2 × BAS). All LH data were log transformed before analysis. The rates of change in LH during the up- and down-slopes of the surge were calculated as the mean proportional change between adjacent samples located between the start and peak of the LH surge or between the peak of the surge and its end, respectively.

To determine whether blockade of the LH surge by the GnRH receptor antagonist in cycle 2 affected subsequent surge generation, up- and down-slopes and peak LH values were compared between cycles 1 and 3 (i.e. the control cycles for each ewe) using paired t tests.

Although Nal-Glu was administered to each animal at a specific time relative to its expected LH surge, as assessed in the control cycles, treatment did not always occur at the anticipated time due to variation among cycles within animals. After LH values were determined, animals were allocated to one of four groups relative to the time of Nal-Glu treatment: 1) before surge onset, 2) during the ascending limb of the surge, and during the 3) first (DESC-A) or 4) second (DESC-B) half of the descending limb of the LH surge. Data obtained from animals treated during first and second halves of the descending limb of the LH surge (DESC-A and -B) were analyzed separately to determine whether GnRH support for the LH surge was necessary throughout the duration of the surge or whether this requirement waxed with time from the peak surge.

To evaluate the response to Nal-Glu treatment in either DESC-A or -B, when LH had already decreasing, the rate of decline (calculated as described above) was compared in two ways by paired t tests: 1) within a given cycle both before and after treatment, and 2) between the treatment cycle and the mean of the two control cycles (cycles 1 and 3) for each ewe.

**Results**

**Exp 1: validation of approach**

The hormonal profiles of a representative ewe during the artificial follicular phase of the control cycle (cycle 1) and the Nal-Glu treatment cycles (cycle 2 and 3) are illustrated in Fig. 1. All five ewes exhibited a LH surge in response to the estradiol rise of the control cycle (Fig. 1, left). Treatment with Nal-Glu before the expected time of surge onset in cycles 2 and 3 completely blocked the LH surge; yet, all animals exhibited a robust GnRH surge as measured in cycle 3 (Fig. 1, right). The time course and shape of the GnRH surges in cycle 3 resembled those previously observed in the artificial follicular phase model under conditions in which antagonist was not given (6, 28). These results indicate Nal-Glu effectively blocks the LH surge without interrupting the GnRH surge. This validates use of the antagonist as an approach to determine how much of the GnRH surge, in terms of duration, is needed for generation of a LH surge of full duration and amplitude.

**Exp 2: duration of GnRH stimulus required for the LH surge**

**Additional validation steps.** In addition to validating the experimental approach in Exp 1, 2 controls were built into the design of Exp 2. First, because the study spanned much of the breeding season (cycle 1 in October and cycle 5 in December), it was necessary to test whether the time during the breeding season affected the outcome of the study. For this purpose, 5 control animals did not receive Nal-Glu in any of the 5 artificial follicular phases (25 total surge inductions). In all instances, an unambiguous LH surge was induced. Trends were noted for increased latency from onset of the estradiol signal to initiation and peak of the LH surge between cycles 1 and 3 (October 12 to November 16), for decreased latency between cycle 3 and 5 (November 16 to December 21), for a slightly increased presurge baseline between cycles 1 and 3, and for decreased baseline between cycles 3 and 5. Across the study period, however, these trends were not statistically significant. Given the objective of this study, we do not consider time within the breeding season to be a factor.

The second additional validation step was to test whether blockade of the LH surge by the antagonist in one cycle affected subsequent generation of the LH surge. For this purpose, none of the 25 animals received antagonist treatment in cycles 1 and 3; Nal-Glu was given during the intervening cycle. All animals in both cycles 1 and 3 exhibited normal LH surges. Figure 2 compares the mean (±SE) LH concentrations during the surges in cycles 1 and 3 for the 20 animals that received Nal-Glu in cycle 2. Neither peak amplitude nor rates of ascent and descent differed significantly between cycles 1 and 3. Thus, blockade of GnRH action during cycle 2 did not affect generation of the LH surge in the subsequent cycle.

**Treatment with GnRH antagonist before the surge.** Nal-Glu treatment was begun before the onset of the LH surge in a total...
of 25 artificial follicular phases. The LH profiles from 3 representative ewes are illustrated in Fig. 3 together with data from 1 of the 2 control cycles in each animal. In every instance, LH decreased after Nal-Glu administration to a level that remained at or near assay sensitivity, and no LH surge occurred. This finding demonstrates that in the ewe, GnRH action is needed not only to sustain basal LH secretion before onset of the surge, but also to induce the LH surge.

**Treatment with GnRH antagonist during the ascending limb of the LH surge.** Antagonist treatment was begun during the ascending limb of the LH surge in 17 artificial follicular phases. Representative results from 3 ewes are illustrated in Fig. 4. In each of the 17 cases, LH concentrations decreased immediately after the injection of Nal-Glu and remained low for the duration of the experiment. Comparison of treatment and control cycles suggests that the antagonist interrupted progression of the LH surge, and this response was not dependent on the time within the ascending limb that Nal-Glu treatment was begun. LH decreased precipitously in animals treated near the start (Fig. 4, cycle 16E) as well as near the apex (Fig. 4, cycle 17D) of the LH surge.

**Treatment with GnRH antagonist during the descending limb of the LH surge.** To test for an effect of Nal-Glu treatment in animals in which LH concentrations were already decreasing, the rate of decline was assessed in 2 ways: comparison of the rate within treatment cycles before and after administration of the antagonist and comparison of the rate in each treatment cycle to an equivalent period of the mean of the 2 control cycles for each individual. Overall, Nal-Glu treatment was initiated during the first half of the descending limb of the LH surge in 6 artificial follicular phases (DESC-A) and in the last half in 10 instances (DESC-B). Patterns of LH in 2 representative ewes of the DESC-A and DESC-B groups are illustrated separately in Figs. 5 and 6, respectively. The calculated rates of decline of the LH surge during treatment and control cycles are compared in Table 1.

Treatment during the first half of the descending limb consistently suppressed LH secretion and led to early termination of the LH surge (Fig. 5). Within treatment cycles, the rate of LH decline increased after Nal-Glu treatment (from 21.8% to 49.7%/h, pre- vs. post-Nal-Glu; P < 0.005; Table 1, DESC-A group). Similar significant differences was noted between equivalent periods of the treatment and control cycles. Namely, although the rates did not differ before Nal-Glu (21.8% vs. 18.5%/h, treatment vs. control cycles), the fall in LH was accelerated after Nal-Glu administration (49.7% vs. 35.8%, treatment vs. control cycles; P < 0.01).

Similar results were observed in the second half of the descending limb of the LH surge (DESC-B group). Nal-Glu again caused premature termination of the LH surge (Fig. 6). As in the animals treated during the first half of the descending limb, the rate of decline within treatment cycles also accelerated after antagonist treatment (from 37.3% to 47.1%/h, pre- vs. post-Nal-Glu; P < 0.01; Table 1, DESC-B group). Further, although the rate of decline between treatment and control cycles did not differ during the pretreatment period (37.3% vs. 34.0%/h, treatment vs. control cycles), LH concentrations fell faster once Nal-Glu was administered (47.1% vs. 28.9%/h, treatment vs. control cycles; P < 0.001).
Discussion

In this study, ewes were treated with a competitive GnRH receptor antagonist at various times relative to the GnRH/LH surge to examine the duration of the endogenous GnRH signal required for expression of a normal LH surge. As GnRH action is a receptor-mediated event, the LH surge should end prematurely if the antagonist were applied while continued GnRH support is needed for progression of the LH surge. Our results demonstrate that blockade of GnRH action at any point during the LH surge, even near the end of the descending limb, caused premature termination of the surge. This supports the conclusion that in the ewe, GnRH action is required throughout the duration of the LH surge for a full surge to occur.

Due to the complex nature of the hormonal feedback systems involved in generation of the LH surge (long, short, and possibly ultra short loop feedback), a crucial aspect of a study such as this is the selection of an appropriate model. Specifically, it is essential to ensure that any changes in the characteristics of the observed LH surge are due to manipulation of the variable of interest, in this case the effective duration of the endogenous GnRH signal, and not artifacts resulting from a more general perturbation of the hypothalamic-pituitary-gonadal axis. For example, both estradiol and GnRH are known to affect pituitary responsiveness to GnRH (1, 29–31). Therefore, both have the potential to influence the characteristics of the LH surge. Thus, it was critical to use an experimental model in which these components are not affected by the experimental treatments. The integrity of the surge-inducing estradiol signal was guaranteed by the use of the artificial follicular phase model (19). In this model, the estradiol signal for the LH surge is maintained, even when endogenous gonadotropin secretion is suppressed by blockade of GnRH action. In intact animals,
such a reduction in gonadotropin output could result in decreased estradiol production, follicular atresia, and abolition or alteration of the preovulatory surges of GnRH and LH. That GnRH secretion was not affected by antagonist treatment was confirmed in Exp 1. Endogenous GnRH surges, with amplitude and duration similar to those previously observed in the artificial follicular phase model (6), occurred in the face of antagonist treatment sufficient to block the LH surge. This confirms the recent finding that Nal-Glu blocks the LH surge in the ewe without interfering with GnRH secretion (32).

The results of previous studies conducted to identify exogenous GnRH treatment regimens capable of inducing a LH surge in the ewe are fully complementary with the present observations. Those studies all indicated that some increment of GnRH, over and above the ongoing high frequency pulses characteristic of the midfollicular phase, are needed for the production of a preovulatory-like LH surge in the ewe (1, 15-18). Nevertheless, a wide variety of GnRH treatment regimens were found to be effective, including increased pulse amplitude or frequency, or large bolus doses of GnRH. Many of these studies were conducted before full characterization of GnRH secretion at the time of the preovulatory LH surge; treatment regimens generally focused on variations in the pulsatile delivery of GnRH. However, the actual time course of GnRH delivered to the pituitary via the portal circulation during the LH surge in the follicular phase model appears to change in a highly complex fashion. Before the surge, the pattern of GnRH release is strictly episodic. At the very start of the LH surge, in either the follicular phase model or the natural follicular phase, GnRH pulses become larger, and secretion between pulses increases markedly. Thereafter, a massive and continuous elevation of GnRH persists for the remainder of the LH surge (4, 33). The results of the previous studies that tested the response to exogenous GnRH, therefore, must be interpreted with caution relative to the elucidation of the physiologically relevant portion of the endogenous GnRH surge, as they did not restore the actual patterns of GnRH secretion seen at the time of the LH surge.

Before the present work, one study did address the question of the active component of the endogenous GnRH signal in the ewe. By administering barbiturate anesthesia (presumably blocking GnRH release) at different times during the period of the LH surge, Webb et al. (13) observed that anesthesia before, but not after, the peak of the LH surge interrupted progression of the surge. They concluded that GnRH stimulation is required throughout the ascending limb of the LH surge but not during the descending limb. Our present findings are not entirely consistent with this conclusion. We found that GnRH stimulation is required not only during the ascending limb of the surge, but also after the peak and even as late as the final portion of the descending limb when LH concentrations approach baseline. Our results agree with the findings of Phillips et al. (17), who reported that the GnRH-induced LH surge in the ewe was curtailed when the delivery of GnRH was stopped before the end of the LH surge. The different conclusions relating to the continued dependence upon GnRH stimulation after the LH peak probably reflect differences in experimental models.

### Table 1. Rate of decline in LH in ewes treated with the GnRH antagonist (Nal-Glu) during either the first (DESC-A) or the second (DESC-B) half of the descending limb of the LH surge

<table>
<thead>
<tr>
<th></th>
<th>DESC-A</th>
<th>DESC-B</th>
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<tbody>
<tr>
<td></td>
<td>Pre Nal-Glu</td>
<td>Post Nal-Glu</td>
</tr>
<tr>
<td>Treatment cycles</td>
<td>21.8 ± 4.5(^b)</td>
<td>49.7 ± 4.9(^e)</td>
</tr>
<tr>
<td>Control cycles</td>
<td>18.5 ± 1.6(^a)</td>
<td>35.8 ± 4.7(^f)</td>
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Values are the rate of decline expressed as the percent drop per h. For each treatment cycle, the rates of decline before and after antagonist treatment were compared within treatment cycles and to those in the equivalent period in control cycles. Within rows, \(^b\) vs. \(^e\) and \(^a\) vs. \(^f\), \(P < 0.005\); \(^d\) vs. \(^e\), \(P < 0.01\). Within columns: \(^\) vs. \(^f\), \(P < 0.01\); \(^e\) vs. \(^g\), \(P < 0.001\).

\(^a\) Nal-Glu was administered only during treatment cycles. In control cycles, post Nal-Glu data are the average rate of decline during the period equivalent to that in which animals received Nal-Glu in the treatment cycles. The increase in the rate of decline in the control animals in DESC-A can be attributed to the proximity of the time of Nal-Glu treatment in these cycles to the inflexion point at the peak.
GnRH SIGNAL FOR LH SURGE

A surge in GnRH signal generates a full LH surge. Studies in women using the same approach as in this study (administration of a GnRH antagonist) lead to a conclusion that GnRH is required for generation of the preovulatory LH surge of the human menstrual cycle. Another study in women compared responses to different doses of Nal-Glu at different periods of the menstrual cycle, including preovulatory stages of the follicular phase, preovulatory gonadotropin surge, and early luteal phase. The antagonist inhibited LH and FSH at all cycle stages, indicating the surge, again suggesting dependence upon GnRH for generation of the gonadotropin surge. Of interest, the effectiveness of Nal-Glu was greatest during the LH surge, a finding that led to the speculation that GnRH secretion is decreased at this time of the human menstrual cycle. It is unfortunate that methods for direct measurement of GnRH in women are not currently available to assess this speculation, because it is at odds with the large increase in GnRH secretion observed by direct measurements in other species, including another primate (2-4, 6-12, 28, 35).

Although the present results allow conclusions relative to the duration of the GnRH signal needed to generate the LH surge in the ewe, they do not address several other important questions related to the massive and sustained release of GnRH that accompanies the LH surge. How much of the GnRH surge in terms of amplitude is needed to produce a full LH surge? Is there a specific qualitative change (e.g. interpulse secretion) in the pattern of GnRH secretion that is responsible for initiating the LH surge? What role, if any, is served by the large quantity of GnRH released after the LH surge has ended? With respect to the amplitude of the GnRH signal, preliminary results from our laboratory suggest that the massive release of GnRH during the surge is far in excess of that required to generate a full LH surge in the ewe (43). Whether there is a specific aspect of the GnRH signal (e.g. a change in pulse amplitude, frequency, or interpulse secretion) that initiates the LH surge has not yet been systematically examined. Nevertheless, the minute to minute pattern of GnRH in the pituitary portal blood of the ewe appears to change from being strictly episodic to continuously elevated at the very onset of the LH surge in either the natural or artificial follicular phase, raising the possibility that sustained stimulation may be critical (4).

With regard to the physiological significance of the prolonged GnRH surge, which can persist for up to 28 h in the ewe (3, 6, 28, 32, 44), the present study attributes function for the first 8–12 h, ensuring generation of a full LH surge. This, however, may be an overestimate of the physiologically relevant duration, as it is not known how much of the LH surge is required for ovulation and development of normal postovulatory ovarian function. Any role for the latter half of the GnRH surge remains unknown. Suggested roles include stimulation of reproductive behavior, the secondary FSH surge, and gonadotropin biosynthesis in preparation for the subsequent gonadotropin surge. Although not directly addressing these points, the present study offers insight into the third possibility. The similarity of the LH surges induced in control cycles 1 and 3 (the latter of which followed Nal-Glu blockade) suggests that the extended portion of the GnRH surge is not required for repletion of gonadotropin stores in preparation for the subsequent gonadotropin surge. A role for restoration of gonadotropins during the intervening luteal phase, however, cannot be ruled out.

A final aspect of the present study is noteworthy. Given the divergence in termination of the LH and GnRH surges and the demonstration that the LH surge does not end because of secretion of a less bioactive form of GnRH (32), it has been postulated that changes at the level of the pituitary gland cause termination of the LH surge. Suggested changes include desensitization to the stimulatory actions of GnRH and/or depletion of secretable LH from the pituitary (32, 45–49). The present results indicate that antagonism of GnRH action during the descent of the LH surge, even near its very end, caused premature termination of the LH surge. Thus, although pituitary desensitization and gonadotropin depletion may well be major factors involved in the demise of the LH surge, at least some GnRH receptors capable of stimulating LH release remain active throughout the full period of the LH surge.

To summarize, the results of this study demonstrate that GnRH stimulation of the pituitary gland of sheep is required throughout the duration of the LH surge for induction of a LH surge of normal amplitude and duration. Blockade of GnRH receptor action before the LH surge or at any point during its ascending or descending limbs terminates further LH release and prevents generation of a full LH surge.

Acknowledgments

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