Dynamics of Gonadotropin-Releasing Hormone Release during a Pulse*

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ABSTRACT. This study examined the nature of the GnRH signal that travels down the pituitary portal vessels and causes an LH pulse. Individual GnRH pulses were described in terms of abruptness of increase and decrease, amplitude, duration, and amount of GnRH released. Pituitary portal blood was obtained at 30-sec intervals for 2.5 or 5 h from five short-term ovariectomized ewes. Jugular blood was sampled every 10 min for LH. We examined 13 GnRH pulses; each produced an LH pulse. The contour of most GnRH pulses approximated a square wave. The rising edge of the GnRH pulse was very abrupt; GnRH secretion increased as much as 50-fold within 1 min. The mean peak amount of GnRH collected during pulses (24 pg/min, range 2-66) was 70-fold greater than the interpulse baseline (0.2-0.5 pg/min). The release period was sustained an average of 5.5 min; thereafter, GnRH fell to prepulse levels within 3 min. Overall, the larger and more prolonged pulses of GnRH were associated with higher amplitude LH pulses. To assess the distortion of the GnRH signal by the collection procedure, samples were obtained in vitro using the same technique during application of 4- and 7-min square wave GnRH pulses by means of a syringe pump. Signals were carried as square-waves through the sampling operation with minimal distortion, with the exception that amplitude decreased during the collection procedure. Our findings indicate the square-wave pulses observed in vivo are an accurate description of the dynamics of GnRH release during a pulse in short-term ovariectomized ewes. (Endocrinology 130: 503-510, 1992)

The precise secretory dynamics of the endocrine neurons controlling anterior pituitary gland function have not been determined directly for any hypophyseotropic substance. Due to methodological limitations, characterization of neuroendocrine function thus far has included mainly indirect measurements, such as monitoring pituitary hormone levels in the peripheral circulation (1), deconvolution analysis to estimate pituitary hormone release (2), and recording of multiunit electrical activity in the hypothalamus associated with pituitary hormone release (3). More recently, direct monitoring of the levels of hypothalamic releasing hormones has become possible using push-pull perfusion (4) or collection of pituitary portal blood (5, 6). These techniques have been applied most extensively in the area of reproductive biology. It has been well established that GnRH is released from nerve terminals in the median eminence in discrete episodes resulting in the pulsatile release of LH from the pituitary (4-7). The exact contour of the GnRH pulse, however, is not known. Characterizing the minute to minute secretion of GnRH during a pulse could be extremely important. For example, recent findings in the ewe indicate pulsatile GnRH secretion varies markedly during the estrous cycle (8, 9). Determining whether these changes in pulsatile secretion include altered shape, duration or amplitude of GnRH release, and whether these parameters are under physiological control, constitutes a new level of analysis of neuroendocrine activity that should enhance our understanding of the neural regulation of reproduction.

The study described here is the first in this line of investigation. Our objective was to characterize the moment to moment changes in GnRH released into pituitary portal blood during individual pulses in short-term ovariectomized ewes, and in so doing, to evaluate the feasibility of using our procedure of portal blood collection for monitoring the dynamics of individual neuroen-
Hormone signals. In this regard, the short-term ovariec-
tomized ewe is known to have robust GnRH signals
which should be amenable to this type of analysis (7).

Materials and Methods

Animals

Studies were performed during the mid-late breeding season
on six adult Suffolk ewes maintained outdoors at the Sheep
Research Facility near Ann Arbor, Michigan (42°18'N). Ewes
were fed a diet of hay and had free access to water and mineral
licks. Sequential samples of pituitary portal blood were ob-
tained from normally behaving conscious sheep using a modi-
fication (7) of the technique of Caraty and Locatelli (6, 10).
The ewes were sampled in pairs to avoid isolation stress. The
apparatus for sampling portal blood was surgically implanted 1
week before collection of portal blood. Surgeries for installa-
tion of the apparatus, and for insertion of steroid implants and
ovariectomy, were performed aseptically under anesthesia as
described previously (7). All procedures were approved by our
University Committee on the Use and Care of Animals.

We chose to sample portal blood shortly after ovariectomy,
rather than in long-term ovariec-
tomized animals, to avoid any
change in the dynamics of a GnRH pulse that might be conse-
quent to the chronic absence of ovarian hormones. Further, to
avoid sampling of portal blood (which requires heparinization
of the ewe) just after abdominal surgery, ovariectomy was
performed at the time of installation of the collection apparatus,
1 week before sampling. From the time of ovariectomy, midlu-
teral phase serum concentrations of estradiol and progesterone
were maintained by sc Silastic implants as described elsewhere
(11, 12). We previously observed that these implants prevent
the postcastration rise in LH and, by 24 h after their removal,
circulating steroid concentrations become undetectable and
high amplitude pulses of LH and GnRH occur at intervals of
approximately 1 h (7). In this study, we monitored the dynamics
of individual GnRH pulses 24 h after removal of the steroid
implants. Ewes were thus the equivalent of short-term ovari-
tomized animals with regard to gonadal steroids.

Portal blood collection

The apparatus for portal blood collection consists of two
stainless-steel cannulae attached to a plastic cup that is ce-
mented in place just rostral to the anterior face of the pituitary
in portal blood traveling down the collection line in 1-4 cm
“blocks” (average volume, 10 μl) separated by air (Fig. 1B).
This separation of blood minimizes mixing and maintains the
resolution of the GnRH signal as it travels through the collec-
tion line (~3-4 min transit time), although some dispersion of
the signal undoubtedly occurs due to tubal adsorption, as well
as the small amount of pooling in the plastic cup (estimated
less than 50 μl). Since only a portion of the portal vasculature
is cut, the pituitary remains functional such that gonadotropin
secretion can be monitored by sampling peripheral blood.

In the present study, we obtained samples of pituitary portal
blood at the maximal frequency judged to be feasible with this
method. Based on mass of GnRH in portal samples from our
previous studies (7), average volume of portal blood easily
obtainable (0.2-0.3 ml/min) and sensitivity and number of
samples that are contained in a single GnRH assay, a 30-sec
sampling interval and a 2.5 h sampling duration was chosen
to allow all samples from a ewe to be assayed together (301
sequential 30-sec samples). Earlier studies in our animal model
indicate one or more GnRH pulses should occur within 2.5 h
(7). In addition to the 2.5 h of intensive sampling, portal blood
was obtained at 10-min intervals for 3.5 h just before or after
the 30-sec samples from these four ewes to determine if any
GnRH pulses were “missed” at either sampling rate, as would
be indicated by an unaccompanied LH pulse. Duration of
sampling in a fifth ewe was extended to 5 h, as the portal blood
volume in the other ewe being sampled simultaneously was
judged to be insufficient for the rapid sampling. This provided
601 portal blood samples which required two separate assays
to quantify GnRH. Mean volume (±SD) of portal blood obtained
at 30-sec intervals from each of the five ewes, determined after
centrifugation to separate plasma and remove air bubbles, was
0.10 ± 0.01 ml, 0.32 ± 0.07 ml, 0.15 ± 0.02 ml, 0.11 ± 0.03 ml,
0.15 ± 0.01 ml. Jugular blood for assay of LH was sampled at
10-min intervals throughout the sampling period from all five
ewes.

In vitro modeling of a GnRH pulse

To assess the degree of distortion of a GnRH signal between
its departure from the portal vessels through its arrival in the
collection tube, we mimicked exactly the set-up used to sample
portal blood, but replaced the ewe with known GnRH signals
provided artificially by an in vitro pulse-delivery system. For
this purpose, the plastic cup of the collection apparatus was
sealed using Parafilm and Silastic adhesive. A 21-gauge needle
was passed through this seal to deliver blood into the cup at
the same location and rate (0.3 ml/min) it would normally flow
into the cup from portal vessels. The needle was attached to a
Y-connector that led via narrow bore Teflon tubing (id = 0.3
mm) to syringes attached to two custom-developed continu-
ously operating syringe pumps. One pump delivered peripheral
blood to which GnRH, bacitracin (final concentration 3 × 10−4
M) and heparin (80 U/ml) had been added just before the
experiment (solution was 98% blood by volume). The concen-
tration of GnRH (357 pg/ml) was similar to that we observe in
portal blood during a high-amplitude pulse (~330 pg/ml). The
other pump delivered the same mixture devoid of GnRH.
Switches between delivery from each pump were computer
controlled so that time error was negligible and there was no
change in flow rate.

In an attempt to mimic the dynamics of GnRH pulses observed in vivo, triplicate square-wave GnRH signals of 4- and 7-min duration each were applied. These artificially produced GnRH "pulses" were separated by 15 min of blood devoid of GnRH. Blood was aspirated from the cup via the lower needle of the collection apparatus using the same peristaltic pump as for collection of portal blood from a ewe. As in the studies in vivo, the volume rate of draw of the peristaltic pump was adjusted to be greater than the delivery of blood into the cup, thus producing "blocks" of blood separated by air during transit through the collection line to sample tube. The pattern of blood and air in the collection line in vitro, the length of the collection line, and the transit time from collection site to sample tube were indistinguishable from that during collection of portal blood from the sheep. Samples were collected at 30-set intervals, fractionated and assayed as for the in vivo study.

Assays

Concentrations of GnRH (7, 13) and LH (14–16) were determined using previously described RIAs. GnRH was assayed in duplicate following methanol extraction of the entire volume of the portal sample when obtained at 30-sec intervals (extracted sample contained 50–400 μl plasma and 500 μl bacitracin) or 750 μl portal sample when obtained at 10-min intervals (extracted sample contained ~600 μl plasma and the remainder bacitracin). Limit of detection (7 assays) averaged 0.07 pg/tube (equivalent to ~0.1 pg/min); intraassay coefficient of variation (CV) averaged 11%. LH concentration was determined in 50–200 μl of plasma. Sensitivity averaged 59 pg/tube of NIH-LH-S12 (2 assays). Intra- and interassay CV for two serum pools were 8% and 6%, and 4% and 1%, respectively.

Data analysis

Values for GnRH are reported as rate of collection (pg per min) rather than concentration as this minimizes potential sources of error in the portal blood collection technique; for example, contamination with nonportal blood or change in flow rate due to head position (7). The concentration of GnRH in portal blood is approximately 5 times the mass of GnRH collected per minute, although this estimate varies depending on hematocrit and volume of each sample of portal blood.

Pulses of LH were determined using the Cluster pulse detection algorithm of Veldhuis and Johnson (17). Size of the nadir and peak clusters were set at 2 points, the t-statistics for significant increases and decreases were 2.612.6. This combination of cluster sizes and t-statistics corresponds to a false-negative rate of ~5%. In this study, there was an obvious one to one correspondence between identified LH pulses and large secretory bursts of GnRH release (elevated by at least 3 SD above assay sensitivity, see Results). The 100% correspondence between pulses of LH and these obvious bursts of GnRH, referred to hereafter as pulses, occurred regardless of sampling interval, indicating no GnRH pulses were missed due either to low sampling frequency (10-min intervals) or low sample volume (30-sec intervals). Only data from periods of 30-sec sampling intervals were evaluated and presented in the Results in terms of dynamics of GnRH release. Each GnRH pulse observed during a period of 30-sec sampling intervals was quantified in terms of three characteristics: 1) total mass of GnRH collected, 2) duration of release, 3) peak rate of GnRH collection during the pulse.

Results

Dynamics of GnRH during a pulse

Each ewe had at least 1 GnRH pulse during the period of 30-sec sampling, and four of the five ewes exhibited at
least 2 pulses. The patterns of GnRH in the four ewes exhibiting more than 1 pulse are illustrated in Figs. 2-5 (ewe with 5 h of frequent sampling in Fig 2). The contour of most GnRH pulses approximated a square-wave. This consisted of an abrupt increase, an elevated plateau of sustained but fluctuating values, and a precipitous drop—although a few pulses did not fit this broad description (Fig. 3, pulse 1).

All GnRH pulses had an extremely steep rising edge. Within 1 min of pulse onset, GnRH increased as much as 50-fold (Fig. 5, pulse 3), and each pulse reached its plateau within 2 min. During the release period of a pulse, GnRH values were continuously elevated and, in some instances, higher values initially were followed by lower values near the end (Fig. 3, pulse 2). The peak amplitude of the pulse averaged 24 pg/min (range 2-66 pg/min) which is ~70-fold greater than the baseline (0.2-0.5 pg/min). The release period was sustained an average of 5.5 min, but this duration was rather variable (range 1.5-8.5 min). At pulse offset, GnRH values plummeted within 3 min to near the interpulse baseline, which remained relatively stable until the abrupt onset of the next pulse.

The total mass of GnRH collected during a pulse averaged 87 pg, with the largest pulse containing 220 pg (Fig. 4, pulse 2). It is important to stress this is the amount collected, not secreted. This amount is an underestimate of the total mass of GnRH released as only a portion of portal blood is removed during the sampling period and not all GnRH is recovered from our sampling system (see next section on in vitro modeling of a pulse).

Although GnRH remained continuously elevated above baseline during a pulse, considerable variability was seen during the release period (e.g. Fig 4, pulse 2). To assess whether this consisted solely of assay variation or included variation in collection and/or secretion rate, 10 aliquots of a standard pool containing an amount of GnRH similar to that during a pulse were extracted and measured in duplicate as a contiguous series in each assay of this study. The average CV of GnRH values during the plateau of a pulse (30%) was double the intraassay CV (15%) for these standard pools, suggesting variation during the plateau stage of a pulse included variation in rate of GnRH collected.

Of the four ewes in which more than one pulse of GnRH and LH occurred during the period of rapid sampling, three ewes had GnRH pulses of sufficiently different size and duration to examine if changes in GnRH-pulse dynamics within the same animal might be related to changes in LH release (Figs. 3-5). The results suggested this to be true. For example, the second GnRH pulse of ewe 5 and ewe 6 was much larger than the first (Figs. 3 and 4) and resulted in a correspondingly higher-amplitude pulse of LH.

In vitro modeling of a GnRH pulse

The 4- and 7-min square-wave GnRH signals produced in vitro were conveyed through the collection system with very little distortion, as illustrated in Fig. 6, which describes results for one each of the triplicate 4- and 7-min signals. For both signal durations, GnRH in collected blood rose from an undetectable interpulse baseline (0.1 pg/min) to a plateau within 0.5-1.5 min. GnRH remained at this plateau for the remainder of the applied signal, such that the interval from first increase through the end of the plateau closely approximated the duration of the applied signal. Duration of the plateau was thus approximately 0.5-1 min shorter than the applied signal, the difference being the time taken to reach the plateau. During the plateau, fluctuations in GnRH level were

![Fig. 2. Pattern of GnRH (pg/min, bottom, closed symbols) and LH (ng/ml, top, open symbols) from a ewe in which portal blood was collected at 30-sec intervals for 5 h. LH was sampled at 10-min intervals. LH pulses are identified by large filled circles.](image)
apparent, despite the application of a constant concentration (Fig. 6). The fluctuations probably resulted primarily from assay variation because the average CV for values during the plateau (14%) was very similar to that of series of 10 identical aliquots of the GnRH-containing pools of blood used to determine the within assay CV (15%). Following the plateau, levels of GnRH dropped to baseline within 2 min. The amplitudes of the measured signal (50–65 pg/min) were only 50–67% of those applied (92–128 pg/min), indicating a substantial loss in GnRH activity during the \textit{in vitro} collection procedure.

In Fig. 7, two additional examples of a GnRH pulse produced in this \textit{in vitro} system (one for each duration) are superimposed upon two pulses produced \textit{in vivo}. The pattern of GnRH collected for the \textit{in vitro} square-wave signal closely matched that of the pulse produced by the sheep, with the exception that the plateau was less variable and the declining phase was more abrupt for the pulse generated \textit{in vitro}.

**Discussion**

The data presented here represent our first attempt to describe the minute to minute secretory pattern of a
releasing hormone from a population of endocrine neurons in vivo, and they represent our initial study in a line of investigations dealing with the regulation and the importance of contour of GnRH pulses in the process of neuroendocrine signaling. An important feature of the portal blood-collection technique used which makes this type of analysis possible is that GnRH is monitored during its sole pass through the pituitary portal vessels. GnRH is not detectable in peripheral blood even during periods of peak secretion due to a combination of short half-life and dilution in the relatively vast volume of peripheral blood. Thus, recirculated GnRH neither contributes to, nor confounds, the observed neurosecretory signal.

Of importance in interpreting the results of any technique to monitor minute to minute neurosecretory changes is a measure of the degree to which the signal is distorted from generation until detection. Both intrinsic biological and technically introduced sources of distortion need to be considered in this regard. Biological sources alter the signal prior to sampling and include diffusion of GnRH from the intercellular space into the vessels, and changes in signal shape during its short passage down the portal vessels to the pituitary. Alterations in the GnRH signal to this point are inherent to the biology of this system. Another biological source of variation, GnRH diffusion from portal blood into the interstitial fluid of the pituitary gland, is avoided to some extent by sampling before this event is completed.

With regard to distortion introduced by the sampling procedure, two types of error and their approximate magnitudes could be examined by our modeling of GnRH pulses in vitro: pulse shape and pulse amplitude. As for shape, there appeared to be very little distortion in the square-wave signal as it traveled through the collection apparatus, line, and pump. Fluctuations in the level of GnRH during the plateau of the in vitro pulse were no greater than assay variation. As to amplitude distortion of the pulses, the rate of GnRH collected was one half to two thirds the rate of GnRH input. Thus, a substantial loss of GnRH occurs as blood travels from the source to collection tube. Two explanations may be offered. First,
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GnRH may be adsorbed by the collection line or lost by the syringe pump used to deliver the signal. In addition, GnRH is likely to be degraded as it travels through the collection line (transit time 3–4 min at room temperature), as it stands in the iced tube containing protease inhibitor prior to freezing (1–2 h after collection), or during freezer storage (assays 3–10 days after freezing).

With the above in mind, we can interpret the patterns of GnRH release during a pulse in short-term ovariectomized ewes. The vast majority of GnRH pulses have a square-wave shape, with rapid onset, period of elevated release, rapid offset, and essentially no GnRH secretion between distinct secretory bouts. GnRH-pulse durations average 5–6 min, a value similar to that suggested by Clarke (18) who obtained samples of pituitary portal blood at 2-min intervals from ovariectomized ewes. During the sustained release period of a pulse, the variation among measured values was far greater than can be accounted for by experimental and assay error. This provides strong evidence for minute to minute changes in the rate of GnRH release even within a single pulse. Some pulses appear to begin with a short-duration burst of greater release before settling to a sustained plateau. The shape and duration of these latter pulses are similar to the pattern of multiunit electrical activity associated with LH release observed previously in ovariectomized rhesus monkeys (3).

GnRH-pulse amplitude must be evaluated with caution for several reasons. Approximately half the GnRH signal may be lost during transit from the collection apparatus to the sample tube as discussed above. Further, only a portion of portal blood is removed and this portion likely varies among sheep; thus, the peak amount of GnRH is an underestimate of the total and likely varies from sheep to sheep. Moreover, because GnRH diffuses from the blood into the interstitial fluid of the pituitary gland as it travels down the portal vessels, sampling sites lower on the pituitary are likely to produce a lower amplitude GnRH signal, a phenomenon we have observed previously (7, 8) Even with these limitations, comparisons of GnRH-pulse amplitude within the same animal should be valid because these factors should be constant.

Within the same ewe, neighboring GnRH pulses were often of markedly different amplitudes, differences that could not be accounted for by assay error. Since pulse duration was also variable, the mass of GnRH released changed substantially from pulse to pulse. Although our data are limited to a few sheep, a relationship between overall size of a GnRH pulse and amplitude of the resulting LH pulse is clearly suggested by our study, with larger and longer GnRH pulses being associated with higher amplitude LH pulses. Changes in GnRH-pulse size as well as subtle differences in GnRH-pulse shape, such as we have observed, have been postulated to alter LH release by the pituitary. For example, Handlesman et al. (19) observed the pattern of GnRH influences the LH response in hypothalamo-pituitary disconnected sheep, a model in which there is no endogenous GnRH release. Specifically, rapid boluses of GnRH elicited higher amplitude LH pulses than the same amount of GnRH over 6, 12, or 18 min. Further, using perifused sheep pituitary cells, McIntosh and McIntosh (20) showed that square-wave GnRH pulses, with their abrupt rising edge, were more efficient at releasing LH than were GnRH pulses with a sloping increase. Given these earlier observations on the pituitary response to GnRH and our current findings on actual GnRH secretory dynamics, mechanisms appear to exist by which the amount of LH released during a pulse can be influenced by factors.
that act solely at the central level to regulate the size and/or contour of the GnRH neurosecretory signal.

This initial description of the dynamics of GnRH release identifies intriguing new avenues for research. One interesting line of questioning is whether gonadal hormones and neurotransmitters alter GnRH release by changing the shape, duration, and/or amplitude of a pulse. Another exciting application of this methodology is determining whether GnRH secretion during the sustained preovulatory GnRH surge (7, 8) is strictly pulsatile or whether it contains an element of continuous release. Answers to these questions will help elucidate how changing inputs to endocrine neurons, such as those releasing GnRH, "sculpts" their resulting neurosecretory output.

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