Evaluation of LH secretory dynamics during the rat proestrous LH surge

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1Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642; 2Departments of Obstetrics and Gynecology and 4Internal Medicine, 5National Science Foundation Center for Biological Timing, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908; and 3Department of Pathology and Laboratory Medicine, University of Wisconsin Hospital and Clinics, Madison, Wisconsin 53792

Hoeger, Kathleen M., Lisa A. Kolp, Frank J. Strobl, and Johannes D. Veldhuis. Evaluation of LH secretory dynamics during the rat proestrous LH surge. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R219–R225, 1999.—The preovulatory luteinizing hormone (LH) surge results from the integration of complex interactions among gonadal steroids and hypothalamic and pituitary hormones. To evaluate changes in LH secretory dynamics that occur during the rat LH surge, we have 1) obtained frequently sampled serum LH concentration time series, 2) used both waveform-dependent and waveform-independent convolution analyses, and 3) independently assessed proestrous LH half-life and basal non-gonadotropin-releasing hormone (GnRH)-dependent LH secretion during the LH surge. Waveform-independent pulse analysis revealed a 24-fold increase in the maximal pulsatile LH secretory rate attained during late proestrus compared with early proestrus. A 15-fold increase was quantified for the mean LH secretory rate. In complementary analyses, we applied a measured LH half-life of 17 ± 2.7 min and a median basal LH secretion rate of 0.0046 µg·l−1·min−1 for convolution analysis, revealing a 16-fold increase in the mass of LH released/burst and more than sixfold rise in the amplitude of the secretory peaks. Evaluation of the approximate entropy of the LH surge profiles was performed, showing an increase in the orderliness of the LH release process during the surge. We conclude that both quantitative (mass/burst) and qualitative (approximate entropy) features of LH release are regulated during the proestrous LH surge.

deconvolution; pulse analysis; rat luteinizing hormone half-life; approximate entropy

The preovulatory luteinizing hormone (LH) surge represents the culmination of a complex interplay of gonadal steroid, hypothalamic, and pituitary hormones. The precise neuroendocrine mechanisms that subserve this massive increase in serum LH are still not well described. Early analysis of the proestrous LH surge focused on characterizing changes in serum LH concentrations and the rate of rise of blood LH levels (1, 2). Further investigations demonstrated that the release of LH in peripheral blood during the preovulatory LH surge remained significantly pulsatile in the rat (5) and monkey (15, 22). The surgelike rise and fall in serum LH concentrations in the sheep seemed to be accounted for by changes in both LH pulse amplitude and frequency (8, 13). The pulsatile release patterns of LH in diestrous and immediately proestrus rats were comparable just before the surge, but the latter abruptly increased during the surge (4). Mechanistically, the massive increase in serum LH concentrations might be explained by increases in LH secretory burst mass and/or frequency, a prolongation of the effective LH half-life of LH, and/or an increase in gonadotropin-releasing hormone (GnRH)-independent (tonic or basal) LH release. The possible contributions of these factors individually and jointly cannot be adequately assessed using conventional pulse analysis, because blood LH concentrations result from the so-called convolved or combined effects of these dynamics (7, 27). A single prior deconvolution analysis of the rat spontaneous proestrous LH surge has been reported using a model-based multiparameter approach to attempt to simultaneously characterize four distinct parameters: the number of secretory bursts, their amplitudes and duration, basal LH secretion, and LH half-life (29). However, this is technically very challenging given the highly correlated nature of the principal measures of interest; namely LH secretory burst amplitude, basal LH secretion rate, and LH half-life (3, 23, 24, 29). Solutions to this technical impasse require independent knowledge of the LH half-life and basal LH secretion rate (7, 25, 27, 28). Alternatively, model-independent calculations of sample LH secretory rates would be valuable to complement an assumed secretory model structure of approximately Gaussian secretory bursts (24). In addition, the regularity or orderliness of LH release can be now assessed via a model-free and translation- and scale-invariant statistic, approximate entropy (ApEn; 6, 16, 17), which should allow insights into the temporal organization of the LH release process during the surge mode of gonadotropin secretion. To these ends, we have reinvestigated the dynamic neuroendocrine mechanisms underlying the rat preovulatory LH surge by 1) independently assessing the LH half-life and GnRH-independent LH release, 2) applying both waveform-independent and -dependent analyses, and 3) estimating the ApEn of LH release on the (first-differenced) LH data.

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Animals. Adult female Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 200–250 g were used. Animals were housed with a 14:10-h light-dark cycle (lights on 0500). This reliably produces an LH surge in the early afternoon of proestrus. Rat chow and water were given ad libitum. Vaginal smears were recorded daily, and only rats showing at least two consecutive 4-day estrous cycles were used. Daily vaginal smears were continued through at least one estrous cycle after the blood sampling procedure to evaluate sampling effect on cycling or, alternatively, animals were killed to evaluate ovulation the day after sampling. In the experiments involving determination of LH half-life, proestrous rats were used as the source of LH and hypophysectomized rats were used as recipients of injected LH serum. At least 10 days elapsed posthypophysectomy before the animals were used. Daily vaginal smears were continued through at least one estrous cycle after the blood sampling procedure to evaluate sampling effect on cycling or, alternatively, animals were killed to evaluate ovulation the day after sampling. In the experiments involving determination of LH half-life, proestrous rats were used as the source of LH and hypophysectomized rats were used as recipients of injected LH serum. At least 10 days elapsed posthypophysectomy before the animals were used in the protocol.

In the experiments evaluating LH secretion after suppression of GnRH release or action, two methods of suppression were studied. Initial suppression of GnRH was performed using intraperitoneal pentobarbital sodium, which has been shown to reliably suppress the LH surge (2). The GnRH antagonist antide was then used for its simplicity and direct action when this compound was available. In an independent evaluation, animals were killed to evaluate ovulation the day after sampling. In the experiments involving determination of LH half-life, proestrous rats were used as the source of LH and hypophysectomized rats were used as recipients of injected LH serum. At least 10 days elapsed posthypophysectomy before the animals were used in the protocol.

Blood sampling procedure. Blood sampling was performed and bolus injections were made via a Silastic catheter in the right external jugular vein, which was placed under metofane anesthesia at least 24 h preceding the sampling time. Animals were housed separately after the catheter placement under similar conditions in the animal facility. The catheter did not restrict the animal’s mobility. Sampling was performed the afternoon of proestrus either from 1200 to 1800 (early proestrus, n = 6) or from 1530 to 2030 (late proestrus, n = 7). Blood (500 µl) was withdrawn every 5 min and replaced by washed red blood cells resuspended in plasma (hematocrit 54%) plus 0.2 ml heparinized saline (50 IU/ml, catheter volume) in a volume equal to that removed. Replacement blood was prepared via a previously described protocol (21). Animals were carefully observed for alteration in normal behavior during the entire sampling period, with food and water available as wanted.

To evaluate LH half-life, serum from proestrous rats was collected at 1700 via cardiac puncture. Serum (vol 2 ml) was injected into hypophysectomized rats (n = 5) via a jugular catheter with samples taken at 0, 1, 3, 5, 7, 12, 30, and 60 min. Blood samples were stored overnight at 4°C, and the serum was separated the following morning and stored at −20°C until hormone analysis. Results of four LH decay curves are displayed in Fig. 1.

Hormone analysis. Serum samples for LH were analyzed in duplicate by a double-antibody radioimmunoassay. First antibody for LH was National Institute of Diabetes and Digestive and Kidney Diseases anti-rabbit LH-S-11, and hormone levels were expressed in terms of rat LH-RP-3(AFP-7187B) standard for LH. Purified hormone, iodinated with 125I by the chloramine-T method, was supplied by Hazelton Laboratories, Vienna, VA. The second antibody was a goat anti-rabbit antibody supplied by Henniger Laboratories. This LH assay has a sensitivity of 0.1 ng/ml for a 100-µl sample, an intra-assay coefficient of variation of 1.2–12.2% (mean 6.8%), and an interassay coefficient of variation of 11%. For deconvolution analyses, LH duplicate variances within any given series were fit as a power function of LH dose (23).

Data analysis. Serum LH concentration versus time series were generated for all rats sampled. LH half-life data in the hypophysectomized rats (n = 5) were analyzed by exponential
curve fitting as previously described (14, 26, 28) using single component LH elimination kinetics. The mean half-life, 17 ± 2.7 (± SE) min, was used in subsequent model-free deconvolution analysis of frequently sampled LH concentration time series in the proestrous rat. A GnRH-independent (time invariant) LH secretion rate was computed from LH concentration time series in rats (n = 5) treated with pentobarbital sodium (n = 2) or antide (n = 3). The median calculated basal LH secretion rate of 0.0046 µg·l⁻¹·min⁻¹, corresponding to a median basal serum LH concentration of 0.113 µg/l, was used in both the model-specific and model-free deconvolution analyses of the LH concentration time series in the proestrous rats.

Initial deconvolution-based evaluation of LH concentration time series was performed using waveform-independent (model free) analysis. Sample secretion rates were calculated using the PULSE2 program developed by Johnson and Veldhuis (7). This algorithm provides an automated method for calculating all sample secretion rates without assumptions concerning the number or shape of the secretory bursts and/or the presence or absence of basal secretion. Program parameters were defined to include fixed values of the proestrous LH half-life as determined directly (above) and GnRH-independent (basal) LH secretion rate (above). Secretion rates are reported as median as well as mean ± SE values, and histograms of sample secretion rates were generated.

A waveform-specific (Gaussian) model of burstlike hormone secretion (26–28) also was applied. Significant secretory bursts were identifiable at P < 0.01 on the assumption of fixed a priori values of the LH half-life and basal secretion rates determined above (14, 26, 28).

ApEn was calculated for m = 1 (window size or vector length) and r = 0.2 SE (filter or threshold) after first differencing of the LH data to remove nonstationarity (6, 16, 17). ApEn was also calculated for the PULSE2 secretion profiles, because such calculated values are detrended by deconvolution (28). Larger ApEn values denote relatively greater irregularity or disorderliness of serial hormone measures, information that is complementary to pulse analysis (above).

Statistical analysis. Because of significant departures from normality for some of the calculated secretory parameters in the deconvolution analysis, nonparametric testing (Wilcoxon's unpaired rank sum two-tailed test) was used to compare measures in the early proestrus and surge profiles. The unpaired Student's two-tailed t-test was used for comparison of maximal LH secretory rates, mean serum LH concentrations, and integrated areas under the serum LH concentration versus time curves. One-way ANOVA was used to compare the mean ApEn of the suppressed, early proestrus, and surge profiles. P values < 0.05 were construed as significant. Data are given as median and means ± SE.

RESULTS

Figure 2 presents a composite histogram of sample LH secretion rates calculated by waveform-independent deconvolution analysis of six early proestrus control rats (Fig. 2, top), seven proestrus rats sampled during the LH surge (Fig. 2, middle), and five GnRH-suppressed rats (Fig. 2, bottom). A comparison of the LH secretory rates in early proestrus and during the preovulatory surge is presented in Table 1. The maximal LH secretory rate in the early part of proestrus was 0.21 ± 0.27 µg·l⁻¹·min⁻¹, whereas the maximal rate during the proestrus surge was found to be 5.1 ± 0.41 µg·l⁻¹·min⁻¹, a 24-fold increase. Correspondingly, a

| Table 1. Comparison of sample LH secretory rates estimated by waveform-independent deconvolution analysis between early proestrus and preovulatory surge profiles |
|---------------------------------|-----------------|-----------------|---|
| Secretory Rate                  | Surge           | Early Proestrus  | P  |
| Maximum                         | 5.1 ± 0.41      | 0.21 ± 0.03     | <0.001* |
| Mean                            | 0.38 ± 0.06     | 0.02 ± 0.01     | <0.001† |
| Minimum                         | 0.005           | 0.005           | NS  |

Values are means ± SE in µg·l⁻¹·min⁻¹. LH, luteinizing hormone.

* t-Test; † Wilcoxon rank sum.
Fig. 3. Individual model-based (multiple parameter) deconvolution analyses of LH concentration-time series during the surge (left) and early proestrus (right). A: fitted serum LH concentration values over time. B: calculated (predicted) LH secretory rates in the same animals. Note different scales on y-axes to accommodate a variable range of observation among animals. One surge profile, which was selected by random assignment, is not shown.
In this study we have reevaluated the temporal mechanisms subserving the massive increase in serum LH concentrations during the preovulatory LH surge by using for the first time both waveform-independent and waveform-specific (multiple parameter) deconvolution analyses. Deconvolution analysis allows one to compute the number, amplitude, duration, and mass of LH secretory bursts, but the simultaneous calculation of LH half-life and basal LH secretion is technically challenging given the highly correlated nature of these parameters (25). Therefore, in the present investigation, we independently assessed the parameters of LH half-life and basal non-GnRH-dependent LH release. These estimates then were applied to analysis of the proestrous LH surge. Waveform-independent (PULSE2) analysis demonstrated a massive increase in the maximal pulsatile LH secretory rate, namely by 24-fold, during the LH surge. The mean LH secretion rate also rose ~16-fold. As a complementary analytic tool, model-specific (Gaussian waveform) deconvolution analysis revealed an increase in the number of secretory bursts per hour of 1.8-fold and an increase in the mass of LH released per burst of 16-fold (with a 6.7-fold rise in the amplitude of calculated LH secretory bursts). This resulted in a 25-fold increase in the pulsatile production rate of LH during the surge. Thus both approaches disclosed similar (24–25 fold) augmentation of pulsatile LH secretion. These changes could be adequately modeled without invoking an increase in the tonic (non-GnRH dependent) secretion of LH or any prolongation of LH half-life.

A single prior deconvolution analysis of the rat proestrous surge, using model-based computer-assisted estimates of LH half-life and tonic non-GnRH dependent LH release, suggested that the massive rise in LH concentrations during the surge might be accounted for, at least mathematically, by multiple mechanisms, including 1) an increase in the frequency, amplitude, and duration (and/or mass) of LH secreted per pulse, 2) a rise in tonic LH secretion, and 3) a prolongation of LH half-life (29). In the present work, we used additional information in the form of an experimentally derived LH half-life and estimates of non-GnRH-dependent (basal) LH release rates. Given such data, the LH surge in the rat could be adequately modeled without a rise in nonpulsatile LH secretion or a change in LH half-life. Indeed, the amount of basal LH release that we estimated would be expected to be a maximal non-GnRH-dependent contribution to LH release during the surge. We have not attempted to quantitate by deconvolution techniques any (putative) GnRH-independent interpulse basal LH release in view of confounding by highly correlated variables [e.g., between simultaneously fitted basal secretion rates and pulse mass (25)]. Rather, we have directly estimated, by GnRH-suppression experiments, residual (non-GnRH dependent) basal LH secretion.

Assessment of the degree of irregularity of secretion or its “orderliness” may reflect the temporal organization and efficiency of the secretory process over time. Quantification of regularity of hormone release has been accomplished recently via the ApEn statistic (6, 16, 17). This concept has been applied to the evaluation of LH and testosterone secretion in men with evidence that aging of the hypothalamic-pituitary axis results in greater irregularity of LH and androgen secretion (17). The possible utility of this calculation arises from putative boundary or transitional states, wherein changes in the orderliness of pulsatile hormone release may mark the onset of pathophysiology. ApEn is a novel statistic that is complementary to PULSE analysis because it detects subordinate patterns in the data. In particular, ApEn is sensitive to short-term variation rather than epochs. ApEn measures the likelihood that runs of patterns that are close remain close on the next

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Surge</th>
<th>Early Proestrus</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Half-duration, min</td>
<td>11.3 ± 1.4</td>
<td>8.7 ± 3.1</td>
<td>NS</td>
</tr>
<tr>
<td>Bursts, no/h</td>
<td>2.0 ± 0.7</td>
<td>1.1 ± 0.7</td>
<td>0.0375*</td>
</tr>
<tr>
<td>Interburst interval, min</td>
<td>30 ± 4.1</td>
<td>52 ± 10.5</td>
<td>&lt;0.05†</td>
</tr>
<tr>
<td>Mass, µg/l</td>
<td>20 ± 4.5</td>
<td>1.2 ± 3</td>
<td>0.003†</td>
</tr>
<tr>
<td>Amplitude, µg·l·min⁻¹</td>
<td>1.75 ± 0.4</td>
<td>0.26 ± 0.9</td>
<td>0.002†</td>
</tr>
<tr>
<td>Production rate, µg·l·min⁻¹</td>
<td>169 ± 15.1</td>
<td>6.7 ± 1.7</td>
<td>0.001†</td>
</tr>
<tr>
<td>Mean, µg/l</td>
<td>16.3 ± 2.6</td>
<td>0.91 ± 0.17</td>
<td>0.001*</td>
</tr>
<tr>
<td>Integrated, µg/l·min</td>
<td>4908 ± 578</td>
<td>230 ± 44</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Table 2. Waveform-specific deconvolution analysis of LH secretion and clearance parameters in early proestrus and preovulatory surge profiles

Table 3. Observed mean ApEn values for LH surge, early proestrus, and GnRH suppressed time series

<table>
<thead>
<tr>
<th>LH Surge</th>
<th>Early Proestrus</th>
<th>Suppressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.963 ± 0.043*</td>
<td>1.151 ± 0.036</td>
<td>1.026 ± 0.055</td>
</tr>
</tbody>
</table>

Values are means ± SD of observed approximate entropy (ApEn) values (1, 20%). * P = 0.024 by ANOVA.
incremental comparison (16). In the present experiments, we evaluated ApEn in (detrended) LH time series preceding and during the surge, as well as in the LH-suppressed state. Our finding of no ApEn differences in the orderliness of LH secretion between early proestrus and the GnRH-suppressed state but greater orderliness during the LH surge indicates that the serial regularity or orderliness of LH release is actually enhanced during the LH surge. In contrast, the orderliness of neurohormone release is disrupted by aging (17) and various tumoral states (6). Increased orderliness likely reflects enhanced coordination among LH release processes during the surge.

This study was not intended to quantitate the amplitude or frequency of hypothalamic GnRH release episodes at the time of the LH surge. Other studies have shown significant increases in the amount of GnRH secreted by the hypothalamus and significant increases in hypothalamic multiunit electrical activity during the surge in various species (2, 9, 13, 20). Whether both the amplitude and frequency of GnRH release rise in all species at proestrus is not entirely clear. Prior studies in primates have demonstrated that fixed doses of GnRH infused at regular intervals will induce an LH surge in hypothalamopituitary-disconnected animals, suggesting that significantly enhanced pituitary sensitivity to GnRH is also physiologically relevant at the time of an LH surge (10). Experience with GnRH infusion in humans with isolated GnRH deficiency also corroborates this finding (19). Estrogen treatment of postmenopausal women amplifies pituitary responsiveness to exogenous GnRH by augmenting GnRH efficiency (rather than potency) (18). Furthermore, there is evident GnRH dependency of the progesterone-induced surge like release of LH in the estrogen-primed postmenopausal woman, as inferred by the ability of pretreatment with a potent GnRH antagonist to abolish the otherwise consistent increase in LH release (11). The present results in the rat show suppression of the proestrous LH surge by a different GnRH antagonist administered immediately before the anticipated LH surge, lending support to the GnRH dependency of the preponderance of LH release within this interval of the spontaneous proestrous surge.

In summary, we have demonstrated that the massive increase in serum LH concentrations that occurs during the rat proestrous LH surge can be accounted for by a 24- to 25-fold increase in the maximal pulsatile LH secretory rate, which by deconvolution analysis is shown to be a result of a 1.9-fold increase in burst frequency and a 16-fold increase in the mass of LH released per burst. These contributing mechanisms can adequately account for the amount of LH released during the surge without invoking a change in the LH half-life or an increase in non-GnRH-dependent LH release. Moreover, the quantifiable regularity of LH release is enhanced during the surge (as estimated by an approximate entropy statistic), indicating an amplification of coordinated LH secretion.

Perspectives

The preovulatory proestrous LH surge, a key event in the estrous cycle, has been the subject of much prior investigation. Although much has been learned about the influence of GnRH and the release of LH, the precise neuroendocrine mechanisms underlying the massive increase in serum LH have not been well described. We have shown that the increase in serum LH can be explained by a 25-fold increase in the production rate of LH, which appears to be dependent on the input of GnRH. In addition, the orderliness of LH release is enhanced during the surge. With respect to disease states, both an abnormal amount of LH production and an increase in disorder in secretion can be potential sources for reproductive abnormalities. A clear understanding of the normal processes that subserve the complex interactions of gonadal steroids and hypothalamic and pituitary hormones that define the preovulatory LH surge is important to our understanding of disease processes. This current work serves to better define the normal process in a single species. Additional work should be undertaken to understand these processes in other species. Furthermore, elucidation of the precise role of GnRH in facilitating the LH surge with regards to enhanced pituitary sensitivity or an increase in the amount of GnRH released as well as the underlying neuroendocrine control mechanisms is needed.

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