Explicating hypergonadotropism in postmenopausal women: a statistical model

DANIEL M. KEENAN1 AND JOHANNES D. VELDHUIS2,3

1Department of Statistics, 2Division of Endocrinology, Health Sciences Center, 3National Science Foundation Center for Biological Timing, University of Virginia, Charlottesville, Virginia 22908

Keenan, Daniel M., and Johannes D. Veldhuis. Explicating hypergonadotropism in postmenopausal women: a statistical model. Am J Physiol Regulatory Integrative Comp Physiol 278: R1247–R1257, 2000.—Neurohormone secretion is viewed here as a variable (unknown) admixture of basal and pulsatile release mechanisms, convolved with individually fitted biexponential elimination kinetics. This construct allows maximum-likelihood estimates of both (regulated and constitutive) components of hormone secretion. Thereby, we infer that a prolonged slow-component half-life of gonadotropin removal and amplified pulsatile (and total) daily luteinizing hormone (LH) secretion rates jointly explicate the postmenopausal elevation in serum LH concentrations without a necessary rise in basal LH secretion rates. This biomathematical formulation should be useful in exploring other neuroregulatory mechanisms that underlie single or dual alterations in the basal versus pulsatile modes of hormone secretion.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.


IN VIVO AND IN VITRO STUDIES of endocrine glands suggest the existence of two physiologically distinguishable modes of regulated secretion, namely, time-invariant basal hormone secretion and secretagogue-driven pulsatile hormone release (15). The basal mode may represent a constitutive, unregulated, or slowly varying hormone release process (3, 12, 19, 34, 39, 40). In contrast, pulsatile secretion likely reflects rapid exocytosis of previously accumulated neurohormone (19, 43).

Pulsatile hormone release was recognized shortly after the development of RIAs in the early 1970s. Subsequent studies revealed that, in some cases, the magnitude and/or frequency of a pulse signal is critical to its tissue actions; e.g., in the gonadotropin releasing hormone-luteinizing hormone (GnRH-LH)-gonadal axis; for insulin secretion and action; in the growth hormone-insulin-like growth factor-1 (GH-IGF-1) axis, and, for the actions of parathyroid hormone (PTH), oxytocin, or glucagon (15, 17, 29, 31, 35, 36, 38, 41). In contrast, knowledge of the mechanisms underlying basal hormone secretion and its tissue impact has lagged considerably. Indeed, in the case of several hormones, such as GH, physiological basal secretion was not demonstrable until the recent emergence of ultrahigh-sensitivity assays (14). On the other hand, apparently elevated basal hormone release is evident in various neuroendocrine pathologies, such as GH, ACTH, and prolactin-secreting pituitary tumors (12) and aldosteronomas (39).

A variable admixture of basal and pulsatile secretion seems to typify the release of some neurohormones, e.g., prolactin, GnRH during the preovulatory LH surge, and PTH, wherein 30–70% of hormone release appears to be nonpulsatile (10, 14, 23, 34).

Admixed basal and pulsatile hormone release also seems to characterize feedback withdrawn states, e.g., hypersecretion of PTH in hypocalcemia (34) or LH in response to testosterone withdrawal (49). Thus accurately partitioning basal versus pulsatile hormone secretion, albeit technically challenging (44, 47), should aid in separating normal physiology (low basal release), pathophysiology (jointly increased basal and pulsatile secretion), and pathology (elevated basal hormone production). To date, reliable segmentation of basal versus pulsatile release has been confounded technically by strong correlations among hormone half-life and basal and pulsatile secretion rates (44, 47). A recent step toward addressing this impasse is nonparametric or waveform-free half-life-dependent deconvolution techniques (7, 16, 24), which to date have received limited or no validated application to extended hormone profiles. As an alternative strategy, here we illustrate a physiologically motivated analytic construct that embodies variably combined basal and pulsatile hormone release, random secretory bursting, and fitted estimates of slow and fast half-lives.

METHODOLOGY

Overall model. Our general formulation defines \( Z(t) \) as the hormone secretion rate at time \( t \) (units for LH = IU \cdot l^{-1} \cdot min^{-1})

\[
Z(t) = \beta_0 + P(t)
\]

where \( \beta_0 \) is the unknown basal secretion rate and \( P(t) \) is the (nonconstant) pulsatile secretion rate. As a disappearance model, we allow for a fast and slow elimination component, where \( a \) and \( 1 - a \) are their respective proportions. In Ref. 21 we show that rate of change in the blood hormone concentration \( X(t) \) is then
described by differential equations, whose solution is

\[
X(t) = [ae^{-at} + (1 - a) e^{-bt}]X(0) \\
+ \frac{\beta_0 a}{\alpha_1} \left(1 - e^{-at}\right) + \frac{1 - a}{\alpha_2} \left(1 - e^{-bt}\right) + \int_0^t \left(e^{-(a+b)t} - e^{-at}\right) X(t) dt
\]

(2)

Here, we add the consideration of biexponential fitting of both the elimination process and estimation of each of three constructs of basal secretion (\(\beta_0\)). The three models are 1) freely varying or analytically fitted (F model, above); 2) zero basal (Z model); 3) basal secretion constrained a) to a known steady-state (C(SS) model) hormone concentration (e.g., measured before experimental hormone injections) or b) to a percentage of total secretion (C(11) model, where \(\gamma\) is a literature-based population parameter (e.g., \(\gamma = 11\%\) of the LH concentration for men). If pulsatile LH secretion is eliminated selectively in equation 2, then the steady-state hormone concentration \([X(t)]\) is the first term. To indirectly estimate basal LH release in models 3a or 3b, we use data from earlier clinical experiments that used GnRH agonists or antagonists to largely eliminate (GnRH stimulated) LH pulses (6, 11, 30). First, we injected a GnRH agonist (leuprolide) to achieve a low known rate of basal LH release, superimposed on which we infused known (pulsatile) amounts of recombinant LH. This experiment identified terms for construct C(SS) above, consisting of a known basal LH. Second, we used our own and literature-based experiments with GnRH antagonists that preferentially disable (GnRH stimulated) pulsatile LH release, thus allowing estimates of (fractional) basal secretion: construct C(\(\gamma\)) above. In addition, on the basis of the published biexponential kinetics of highly purified human LH in hypopituitary volunteers (46), we further validated quantitative model recovery from distribution volume estimates. Clinical methods. Serum immunoreactive LH concentration time series were obtained and reported previously by sampling blood in healthy women at 10-min intervals for 24 h, after provision of written informed consent approved by the Human Investigation Committee (8, 32). Six volunteers were studied in each of the following categories: for each of three separate phases of the normal menstrual cycle [early follicular (EF), late follicular (LF), and midluteal (MI) phases] and estrogen-unreplaced postmenopausal women (ages 55–75). The ranges of the intra- and interassay coefficients of variation in the LH immunoradiometric assay (IRMA) are 4.5–8.3% and 6.8–10%, and assay sensitivity is 0.8 IU/l (First International Reference Preparation).

As one biological validation strategy, we administered the GnRH agonist, leuprolide acetate (3.75 mg im), to downregulate endogenous LH release in five healthy men. Three weeks thereafter, volunteers received intravenous bolus injections of human recombinant LH (Serono Laboratories, Norwell, MA) at 2-h intervals. LH was infused at a fixed dose of 7.5, 15, 30, 50, or 75 IU/pulse over 1 or 8 minutes for a total of four to eight consecutive infusion pulses. Blood was withdrawn at 10-min intervals beginning immediately before the first infused LH pulse (0800 clock time) and throughout the infusion until 3 h after the last injection. Serum was later assayed for LH content by IRMA (above). Preinjection serum testosterone concentrations were <85 ng/dl (2.5 nmol/l) during leuprolide-induced suppression of the gonadotropin axis.

RESULTS

In the five LH-suppressed men infused with variable but known amounts of recombinant human LH, we estimated the several masses of LH infused (known amounts were 7.5, 15, 30, 50 and 75 IU/pulse; Serono). To this end, we applied each of the three separate formulations of "basal" LH secretion: 1) freely varying or analytically fitted (F model); 2) zero basal (Z model); 3a) constrained by the preinjection steady-state (C(SS) model) measured serum LH concentration or 3b) constrained by a percentage of total secretion (C(11) model: e.g. 11% for men (30)]. For any model, the calculated slope of the plot of infused LH dose (IU) versus estimated LH pulse mass (IU/l) is the reciprocal of the LH distribution volume, which was determined independently earlier (46). As illustrated in Fig. 1, the slopes for the four models predict LH distribution volumes of 3.7 (F model), 4.2 (Z model), 4.6 [C(SS) model], and 4.8 liters (C(11) model). The values fall within the estimated adult male range of 3–5 liters based on highly purified human pituitary LH extracts (46), although this could vary depending on LH isofoms and/or gender. The application of all four models of freely varying, zero, and constrained (steady state and 11%) basal secretion is shown. Figure 2 illustrates that, in

![Fig. 1. Plots of linear fits of dose (mass) of recombinant human luteinizing hormone (LH) injected (IU) vs. calculated mass (IU/l) of LH recovered in 5 leuprolide-suppressed men given 1 or 8 min intravenous infusions of 7.5, 15, 30, 50, or 75 IU of LH. LH distribution volume (V0) is estimated by reciprocal of slope.](http://ajpregu.physiology.org/).
Recombinant Human LH Infusions

Fig. 2. Plots of mass of human recombinant LH infused vs. first (rapid)- or second (slow)-phase LH half-lives (\(t_{1/2}\)) calculated for steady-state constrained (pre-LH injection) model of basal LH secretion for experiment defined in Fig. 1. Linear regression analyses are shown with corresponding correlation coefficients and \(P\) values in 5 men evaluated.

The experimentally known basal [C(SS)] model, the second (slow)-component LH half-life is proportionate \((r = +0.93, P = 0.02)\) to the dose of recombinant human LH injected. Serum LH time series in women sampled every 10 min over 24 h were analyzed next according to the foregoing three formulations of basal secretion: freely varying, zero, or constrained. On the basis of earlier published GnRH antagonist studies in women, constrained basal secretion rates (basal secretion as percentages of total secretion) were taken as nominally 24% in premenopausal females (EF, LF, and ML, respectively) and 34% in postmenopausal women (6, 11). ANOVA applied to the resultant data revealed several major points summarized in Fig. 3, A–E, which shows slow-component LH half-lives; LH pulse frequency; and daily basal, pulsatile, and total LH secretory rates. Illustrative individual women’s data (parameter estimates with corresponding estimated SEs) are given in Tables 1–3.

An unexpected finding was a high within-subject agreement (low variability) among estimates of LH pulse mass (and daily pulsatile LH secretion) across the various basal-secretion models. For example, the median (and range) coefficients of variation (SD/mean \(\times 100\%\)) among the four model-based estimates of LH burst mass within subjects were 3.2 (1.5–9.2%) in EF, 5.4 (3.8–14%) in LF, and 5.8 (2.3–16%) in ML phase young women and 4.5 (1.7–5.0%) in postmenopausal women.

Second, according to all three models, daily pulsatile (and total) LH secretion was maximal in postmenopausal women, and minimal in the ML phase of the menstrual cycle (Fig 3A). Third, two-component LH half-life values fell within the published normal range in the human, namely, for the (freely varying) rapid and slow components, respectively, 7–36 and 46–240 min (8, 46; Fig 3B). These estimates in women compare with (mean \(\pm\) SD) values of 18 \(\pm\) 5 and 90 \(\pm\) 22 min reported earlier for rapid- and slow-phase LH half-lives in four LH-injected hypopituitary men (46). The corresponding LH half-life ranges were 7–25 (rapid phase) and 58–130 min (slow phase) in the C(SS) model using literature-based GnRH-antagonist data to estimate the percentage basal.

In both non-zero basal models, the calculated percentage basal LH secretion ranged absolutely from 1 to 54% within the four groups of woman studied here (see Tables 1–3). In postmenopausal women, percentage basal LH release (variable, analytically estimated) was 2–52%, similar to the values in younger women (1–54%). Thus (in the non-zero basal models) increased absolute basal LH secretion (but not greater fractional partitioning of basal versus pulsatile LH release) characterized post (versus pre-) menopausal women (Fig. 3C).

The highest daily total LH secretion rates (freely varying basal model) were attained in older women (260–980 IU \(\cdot\) l\(^{-1}\) \(\cdot\) 24 h\(^{-1}\)). The range of values in LF phase premenopausal women was 47–850 IU \(\cdot\) l\(^{-1}\) \(\cdot\) 24 h\(^{-1}\), in the EF phase was 63–140 IU \(\cdot\) l\(^{-1}\) \(\cdot\) 24 h\(^{-1}\), and in ML phase was 25–150 IU \(\cdot\) l\(^{-1}\) \(\cdot\) 24 h\(^{-1}\) (Fig. 3D).

On the assumption of zero basal LH secretion (daily) LH pulse mass estimates remained remarkably similar to those in the two other models (freely varying vs. literature-constrained basal secretion). In the zero basal model, estimated second-component LH half-lives also tended \((P = 0.05\) by ANOVA, \(P < 0.05\) by the Kruskal-Wallis test) to be longer in postmenopausal women and shorter in ML phase young women (Fig. 3B).

Use of a literature-constrained (GnRH antagonist predicted) percentage basal rate of LH secretion sometimes predicted lower basal secretion than freely varying (fitted) estimates and lower slow-phase LH half-lives than the zero basal model. The calculated daily LH pulse mass was similar to those in the other two models (Fig 3A). Pulse frequency estimates were model independent and showed slowing only in the ML phase (Fig. 3E).

Illustrative observed 24-h serum LH concentration profiles, calculated (fitted) profiles, and estimated pulsatile and basal LH secretion curves are given in Fig. 4 for each of the three different formulations of basal LH release. The individual profiles highlight 1) variability among menstrual contexts and 2) distinctions among the three analytic constructs of basal (LH) secretion.

As a general modeling comment, which is applicable to Tables 2 and 3, for those parameters that describe the pulsatile structure, mass accumulation (\(\eta_0\), \(\eta_1\)), pulse shape (\(\beta_1, \beta_2, \beta_3\)), and the variance of pulse mass random effects (\(\sigma^2\)), the precision of their estimators is determined as much by the number of pulses as by the actual number of observations. In Tables 2 and 3, illustrating the individual parameter estimates for an EF female and a postmenopausal female, the number of pulse times were 10 and 16, respectively. In Table 2, particularly, some of the (estimated) SEs are large enough that several of the above parameters could not be individually rejected from being zero. In part, this is the consequence of allowing for the flexibility necessary for describing the broad range of profiles encountered in practice; for example, sometimes two of the three
pulse shape parameters will suffice, but which two of the three can vary among individuals, and, in some women, there appears to be large basal secretion, whereas for others there is not. The modeling implication of all of this is somewhat dependent on one's specific goal. If the purpose is to find the most parsimonious model for a particular individual, then one might attempt to eliminate potential parameters in a manner analogous to stepwise regression. However, in the present context, these structural parameters are, in some sense, more like nuisance parameters, whose importance rests in their combination allowing one to soundly estimate total daily pulsatile secretion and to assign a justified SE (Table 1). These resulting aggregates are in general well separated from zero.

DISCUSSION

We here present, validate, and then apply a random-effects and maximum-likelihood methodology to evaluate hormone biexponential elimination and pulsatile and basal neurohormone secretion according to different renditions of basal secretory behavior. This new analytic formulation extends our earlier efforts by 1) delineating three distinct possible notions of basal secretion; 2) implementing biexponential kinetics; 3) requiring independent biological validation using recombinant human LH infusions; and 4) analyzing full 24-h-long serum LH concentration profiles in women in different menstrual contexts to clarify normal physiology. Indeed, evaluating LH secretion in a relatively
large cohort (n = 24) of healthy pre- and postmenopausal women unmasked an unanticipated, remarkably robust within-subject uniformity of estimates of LH secretory burst mass across the three analytically distinct strategies for defining basal gonadotropin release: 1) zero basal and thus purely pulsatile LH secretion; 2) freely varying analytically estimated basal LH secretion; or 3) constrained or putatively non-GnRH-dependent basal LH release. In particular, in any given menstrual context, the three structurally distinct assumptions regarding basal LH secretion predicted a similar mean mass of LH secreted in pulses for any woman. The median within-subject coefficients of variation of estimated LH pulse mass among the three models fell within the range of 3–6%. This primary observation suggests the thesis that, for a fitted two-compartment LH secretory analytic construct, conditional on determinable LH pulse frequency and two-component LH half-lives.

Model-independent within-subject estimation of LH pulse mass has several important implications. First, the pulsatile mode of LH secretion, presumptively activated by hypothalamic GnRH impulses, should be a more robust measurable endpoint of neuroendocrine regulation. Second, unlike our own and other earlier highly parameterized constructs (21), the present more parsimonious formulation of basal and pulsatile LH secretion along with biexponential kinetics obviates some of the strong mathematical confounds that impede maximum-likelihood estimates (44). Third, because the sum of the estimated random mass effects within any given time series is (theoretically) zero (21), we can calculate by maximum-likelihood analysis statistically valid confidence intervals for pulsatile and basal LH secretion and LH half-lives. We showed earlier that this cannot be accomplished reliably for a one-component disappearance idiom (21, 44). Fourth, by comparing basal and pulsatile secretion of LH among reproductive states in healthy women, we could examine gonadotropin regulation across the normal adult human female lifetime.

Table 1. Individual parameter estimates and their SEs for LH secretion and elimination in EF female 1

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>10</td>
<td>160 (130.0)</td>
<td>79 (31.0)</td>
<td>160 (11.0)</td>
<td>63.0 (24.0)</td>
<td>20.0 (9.2)</td>
<td>6.3 (2.4)</td>
<td>10.0 (7.0)</td>
<td>55.0 (18.0)</td>
</tr>
<tr>
<td>Z</td>
<td>61.0 (17.0)</td>
<td>0</td>
<td>61.0 (17.0)</td>
<td>0</td>
<td>6.1 (1.7)</td>
<td>9.9 (5.4)</td>
<td>78.0 (17.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (24)</td>
<td>86.0 (31.0)</td>
<td>20.0 (7.4)</td>
<td>65.0 (24.0)</td>
<td>24</td>
<td>6.5 (2.4)</td>
<td>9.8 (6.1)</td>
<td>51.0 (13.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Individual parameter estimates and their SEs for LH secretion and elimination in EF female 1

<table>
<thead>
<tr>
<th>F</th>
<th>Z</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>β₀</td>
<td>β₁</td>
<td>β₂</td>
</tr>
<tr>
<td>α₁</td>
<td>α₂</td>
<td>β₃</td>
</tr>
<tr>
<td>γ₁</td>
<td>γ₂</td>
<td>β₄</td>
</tr>
<tr>
<td>ω₁</td>
<td>ω₂</td>
<td>ω₃</td>
</tr>
<tr>
<td>φ₁</td>
<td>φ₂</td>
<td>φ₃</td>
</tr>
</tbody>
</table>

Values are means ± SE. β₀, constant, relating rate of LH pulse mass accumulation to prior interpulse interval (IU·l⁻¹·min⁻¹); β₁, β₂, β₃, secretory pulse shape parameters of generalized gamma function (Eq. A1); ω₁, variance of experimental sampling and assay; ω₂, variance of pulse mass. φ₀, basal secretion rate (IU·l⁻¹·min⁻¹); ω₂, rate constant of slow LH elimination (min⁻¹); γ₀, residual mass of LH accumulation since last pulse (IU/l).

Table 3. Individual parameter estimates and their SEs for LH secretion and elimination in PM female 1

<table>
<thead>
<tr>
<th>F</th>
<th>Z</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>β₀</td>
<td>β₁</td>
<td>β₂</td>
</tr>
<tr>
<td>α₁</td>
<td>α₂</td>
<td>β₃</td>
</tr>
<tr>
<td>γ₁</td>
<td>γ₂</td>
<td>β₄</td>
</tr>
<tr>
<td>ω₁</td>
<td>ω₂</td>
<td>ω₃</td>
</tr>
<tr>
<td>φ₁</td>
<td>φ₂</td>
<td>φ₃</td>
</tr>
</tbody>
</table>

Values are means ± SE.
R1252 LH SECRETION IN WOMEN

- LH conc. (U/L) vs. time (min)
- LH secret. rate (U/L/min) vs. time (min)
- LH secret. rate (U/L/min) vs. time (min)

Graphs showing LH secretion patterns over time.
Comparison of LH profiles disclosed that postmenopausal women have a pronounced augmentation of LH secretory burst mass. In principle, such unleashing of pulsatile LH release in the gonadoprival state may reflect an expanded gonadotrop-cell secretory capacity and/or enhanced endogenous GnRH drive (8). In contrast, in premenopausal women, LH pulse mass remained nearly constant across the menstrual cycle, except for the ML phase.

Half-life variations might occur in postmenopausal versus premenopausal women. In particular, according to the zero-basal and constrained-basal models, the calculated LH half-life rises postmenopausally and falls in the ML phase. The former inference was suggested recently in GnRH antagonist-based studies (37). In this regard, a recent kinetic analysis of 14 human LH isoforms in a heterologous assay disclosed up to a twofold range in their in vivo half-lives (3). Analogously, in the human and animals, gonadal status (e.g., postovariectomy) may alter evident rates of in vivo LH disappearance (1, 3, 5, 27, 47, 49, 50).

Postmenopausally increased basal LH secretion was implied only in the constrained basal model. This is in accord with the literature-based inference that the percentage of basal LH secretion, when defined as non-GnRH dependent, is elevated after a GnRH antagonist in postmenopausal women (6, 11, 30). In contrast, according to a fitted basal secretion model, the absolute, but not percentage, basal LH secretion rate is higher in postmenopausal individuals. In neither construction was the variation in basal LH secretion rate the dominant mechanism regulating total daily LH secretion and hence mean serum LH concentrations in premenopausal women. Because estrogen concentrations vary remarkably across the menstrual cycle, these analyses allow one to conjecture further that estrogen or one of its gonadal covariates controls primarily pulsatile (rather than basal) LH secretion. Estrogen may also influence the slow-component half-life of LH, because the latter is elevated in the face of estrogen withdrawal. Whereas clinical studies with GnRH antagonists have suggested a lower percentage of ostensible GnRH-independent LH secretion in young versus older women (6, 11, 30), such measures are derived in the face of near-maximal inhibition of endogenous GnRH action and thus, by definition, do not reflect the interpulse rate of basal LH release that actually occurs in the presence of physiological endogenous GnRH drive. Indeed, GnRH itself might contribute in part to maintaining some fraction of basal or nonpulsatile gonadotrope secretory activity. In fact, our freely varying fitted basal secretion construct predicts higher LH interpulse secretion than that suggested by GnRH antagonist studies.

The present strategy of partitioning total daily hormone release into respective basal and pulsatile components, conditional on pulse times and with the inclusion of random LH pulse-mass effects, simplifies several earlier numerical deconvolution approaches (44). Here we estimate approximately seven principal parameters of hormone secretion and two of hormone elimination, rather than 20–30 parameters as required in some previous efforts (8, 44). The core estimates include basal secretion (βb1); the average rate of interpulse hormone (mass) accumulation (νb1) and a constant (νb2) relating this value to the prior interpulse interval; (one or more of) three key features of the secretory burst shape: rate of secretory burst ascent, peakedness, and steepness of descent; a random-effects term, defining stochastic variability in pulse mass accumulation; and the rapid versus slow hormone half-lives. If one used an a priori hormone secretory pulse shape, e.g., as determined by direct venous catheterization (2), this knowledge would eliminate the fitting of pulse-shape parameters, allowing further simplification. Analogously, independent ascertainment of elimination kinetics would obviate the need to solve for these terms in the analysis. Importantly, the foregoing core parameters all mirror basic physiological processes. For example, the rapid increase and slow decrease in secretion rates within a burst would likely correspond to prompt exocytotic discharge of prestored hormone granules and delayed de novo biosynthesis and release of additional hormone, respectively (19). Similarly, the slow component of hormone (LH) removal would seem to reflect its irreversible metabolic clearance from the body (3, 46).

These analyses uncover other challenging issues in appraising basal hormone secretion. First, absolute basal LH secretion rates can vary by severalfold across the reproductive life span and among individuals. Second, independent knowledge of rapid and slow hormone half-lives would aid in distinguishing the contribution of basal secretion from that of the slower half-life to the mean hormone concentration. In addition, accurate a priori defined hormone half-lives could help to discriminate between otherwise statistically comparable models of combined pulsatile and basal secretion. In contrast, we show here that estimation of the pulse mass of hormone secreted is largely basal model free. Because kinetic values are not always easily established for an individual in any particular clinical or experimental setting, we can suggest in the future also implementing a Bayesian modification of the present approach with preconditioning on the expected population hormone half-life and/or anticipated basal secretion rate.
The present LH time series were collected by blood withdrawal every 10 min for 24 h and show good precision in the estimation of daily LH secretion and several key secretory parameters, as well as the more prolonged half-life phase (see Table 1–3). Pulse shape is less well estimated, in view of relatively infrequent sampling compared with the brevity of LH secretory pulses. Similarly, the rapid half-life component can only be estimated with an adequate sampling interval, \( \Delta t \). That is, the half-life cannot be less than \( \log(2) \times \Delta t \); thus, for \( \Delta t = 10 \) min, the lower bound is 6.93 min.

Although the random-effects maximum-likelihood appraisal of basal and pulsatile LH secretion is illustrated here only in healthy women, this analytic strategy should have relevance in other contexts, e.g., in assessing LH pathophysiology in various study populations and in evaluating the secretory control of other neurohormones. In addition, by incorporating relevant physiological linkages and dose-response interfaces, e.g., as suggested earlier for the integrated male GnRH-LH-testosterone feedforward and feedback axis (20), this core construct might be extended to allow appraisal of joint secretion by coupled glands within an interconnected endocrine network.

Perspectives

The challenge of correctly partitioning an unknown admixture of episodic and basal (nonpulsatile) neurohormone secretion into the two corresponding contributions has been difficult to surmount analytically (44). Here, we extend the notion of dissecting comingled pulsatile and nonpulsatile hormone release given serial measurements of their combined output convolved with elimination processes in peripheral blood. Critical factors making such estimates possible include first the introduction of a biexponential (and hence 2 compartmental) disappearance function, which incorporates several unobserved features of the dissipation phenomenon. Second, both the number and cross-correlated nature of secretion parameters are restricted in the analysis by conditioning the deconvolution solution on predetermined pulse times estimated independently (18). Available a priori knowledge of the biexponential kinetics or the percentage admixture of pulsatile and basal components in the release process would aid further. However, major issues remain unresolved. For example, to date there are few objective in vivo validation strategies that combine constant neurohormone infusions with superimposed pulselike injections of varying defined amplitudes (33). Analogously, more studies are needed that sample blood at high frequency and over a prolonged duration near the site of neurohormone outflow to monitor the patterns of joint basal and pulsatile secretion directly (4, 9, 26, 28, 42). Moreover, more refined analyses will need to incorporate the nonlinear impact of one or multiple binding proteins in plasma and/or tissues on the partitioning of hormone removal (47a, 48). Indeed, empirical biexponential kinetics oversimplify the true physics and physiology that presumably underlie the hormone dispersal process in the circulatory tree. For example, one might envision rapid postsecretory diffusion of molecules in the aqueous space of blood, high-velocity advection along the primary direction of flow in the arterial and proximal venous systems, geometric dilution of hormone molecules by the arborizing vasculature, capillary transit, and secondary confluence into proximal venous trunks. Within this whole body circuitry, there is the region-specific irreversible removal of hormone molecules (e.g., in liver, spleen, kidney, etc.) at some probability level. We reason that an empirically estimated biexponential elimination model approximates the aggregate of these nonlinear processes. Another strategy to be considered will be the simultaneous analytic evaluation of two or more physiologically interlinked hormone series. Joint analyses could allow for more statistically reliable reconstruction of secretion and removal processes, while incorporating the necessary feedback and feedforward relationships between the hormones. For example, this concept might be implemented in relation to corelease of LH and testosterone (men) and LH, progesterone, and/or estradiol (women). In addition, enhanced understanding of the cellular mechanisms that govern constitutive versus regulated facets of nonpulsatile (basal) hormone secretion should also be useful. In principle, combining several of the foregoing strategies could allow insights into and predictions about the dynamic behavior of unobserved signals within the feedback system, such as the hypothalamic release of GnRH in the case of threefold interrelated GnRH, LH, and sex steroid feedback regulation. Therefore, continuing interdisciplinary efforts in integrative physiology and supporting analytic tools should engender continuing new insights into the operating properties of more complex neurophysiological networks. Indeed, more comprehensive biomathematical constructs should eventually encapsulate the full array of functionally interdependent processes within a macroscopic physiological axis, including the intracellular biosynthesis and release of secretory molecules; their postsecretory association with transport proteins; intravascular aqueous diffusion, rapid advection, geometric dispersion, partial recirculation and site-specific saturable removal from the bloodstream; time-delayed feedforward actions on selected target tissues; and the subsequent feedback-controlled synthesis and secretion of new effector molecules.

APPENDIX

Structural Features of the Basal Secretion Models

General structure of secretory formulation. As defined in equation 1 (METHODOLOGY), \( \beta_0 \) is the basal and \( P(\cdot) \) the pulsatile hormone secretion rate. We reason that the cellular basis for basal secretion is constitutive hormone release and for pulsatile secretion is the discharge of available intracellular (peptide) hormone contained within secretory granules, which accumulate during the interpulse interval. Hormone molecules synthesized in the cell starting at pulse time \( T_j^{-1} \) are stored until the next pulse time, \( T_j \). As reviewed in Refs. 8, 19, and 47, several techniques have been validated to estimate GnRH-LH pulse times. Here, we evaluate secretion conditional on the pulse times, as determined in Ref. 19. We define
the mass of the $j$th pulse $M_j$ to be the amount of hormone accumulated between pulse times $T_{j-1}$ and $T_j$. Here, we assume that the LH pulse masses are given by

$$M_j = \eta_0 + \eta_1 \times (T_j - T_{j-1}) + A_j,$$

where $\eta_0$ is the basal (intracellular) accumulation rate, and $\eta_1$ is a constant, which relates pulse mass accumulation to the immediately preceding interpulse interval length. This relationship reflects the fact that gonadotrope cells accumulate more LH during prolonged interpulse intervals (8). We assume that the $A_j$s are independent and identically distributed normal random variables with mean zero and variance $\sigma^2_A$, thus allowing for stochastic variability (random effects) in pulse mass. To accommodate variability skewness pulse shape, a function $\psi(s)$ is specified, which is the normalized rate of secretion given as hormone mass per unit distribution volume per unit time, as a Generalized-Gamma family of densities (i.e., normalized to integrate to 1)

$$\psi(s) = \frac{\beta_1}{\Gamma(\beta_2) \beta_2^{\beta_1}} s^{\beta_1-1}e^{-(s/\beta_2)^{\beta_2}},$$

(A1)

where $\beta_1 > 0$, $\beta_2 > 0$, and $\beta_3 > 0$ are three parameters that delimit the secretory burst shape. The resulting overall secretion rate $Z(t)$ is thus given by

$$Z(t) = \beta_0 + P(t) = \beta_0 + \sum_{j=1}^{\#} M_j \psi(t - T_j).$$

In the case of two elimination components, the foregoing formulation results in equation 2 (Methodology). Infusions of human pituitary LH have suggested that the rapid LH half-life component is approximated by $a = 0.63$ and a half-life of $18 \text{ min}$ [$\alpha_1 = \log(2)/18$] and the longer half-life component by $(1 - a) = 0.37$ and a half-life of $90 \text{ min}$ [$\alpha_2 = \log(2)/90$] (46). Here, we initially fix $a = 0.63$ for the kinetic estimates.

What is then observed is a discrete time sampling of this process, plus measurement error

$$Y_k = X(t_k) + \epsilon_k \quad k = 1, \ldots, n,$$

where $\epsilon_k$s represent measurement error (e.g., due to assaying). In Refs. 21 and 51, the asymptotic normality is shown for the maximum-likelihood estimators of the above parameters $\beta_0, \alpha_1, \alpha_2, \eta_0, \eta_1, \beta_1, \beta_2, \beta_3, \sigma_\alpha, \sigma_\beta$. More importantly, their variances and covariances are estimable, as well as variances and covariances for such constructions as total daily secretion and its partition into total daily basal secretion and total daily pulsatile secretion. For example, to calculate total daily LH secretion, we integrate the (reconstructed) LH secretion rate, $Z(t)$, from 0 to 1,440 min

$$\int_0^{1,440} Z(t) \; dt = \int_0^{1,440} \beta_0 \; dt + \int_0^{1,440} P(t) \; dt.$$

Total daily secretion = total daily basal + total daily pulsatile

$$= \beta_0 \times 1,440 + \int_0^{1,440} P(t) \; dt.$$

Models of basal secretion. Given the foregoing, we next consider three models for basal secretion. In our original construction (19, 21, 51), basal secretion was free to vary ($\beta_0$ was unconstrained) and we allowed a single exponential elimination process. The three models evaluated here are 1) freely varying, i.e., analytically fitted (F model); 2) zero basal (Z model); 3a) constrained by preinjection steady-state [C(SS) model] measured serum LH concentration; or 3b) constrained by a percentage of total secretion [C($\gamma$) model, where $\gamma$ is a literature-based population parameter]. The zero basal (Z) model is a minor adaptation of the estimation methodology for that of the freely varying basal model; there is one fewer parameter (no basal term: $\beta_0$), and the modifications necessary for the appropriate formulas are minor. SEs can be calculated for the parameter estimates and for such constructions as total daily secretion, mass per pulse, etc. These standard errors are given in Tables 1–3.

The constrained basal (C) model is, however, slightly different, and below we describe its framework briefly. In the constrained basal (C) model, the proportion of (daily) basal secretion to total (basal plus pulsatile) secretion is assumed to be constrained; let $\gamma$ be the proportionality constant, a literature-based population parameter. On the basis of published estimates, for premenopausal women $\gamma$ was assumed to be ~24%, and for postmenopausal women, 34%. This model differs from the preceding, because the constraint is global. For example, total daily secretion, total daily basal secretion, and the basal rate $\beta_0$ are now functions of total daily pulsatile secretion

$$\int_0^{1,440} \beta_0 \; dt = \gamma \int_0^{1,440} Z(t) \; dt \quad \text{and} \quad \int_0^{1,440} Z(t) \; dt = \frac{1}{1-\gamma} \int_0^{1,440} P(t) \; dt,$$

hence

$$\beta_0 = \frac{\gamma}{1,440 (1-\gamma)} \int_0^{1,440} P(t) \; dt$$

$$= \frac{\gamma}{1,440 (1-\gamma)} \sum_{t=1}^{T_j} [\eta_0 + \eta_1 \times (T_j - T_{j-1})] \psi(t - T_j) + \frac{\gamma}{1,440 (1-\gamma)} \int_0^{1,440} \sum_{t=1}^{T_j} A_j \psi(t - T_j) \; dt$$

= a deterministic part + a random part

The basal rate $\beta_0$ is now a random variable, as a consequence of the random effects: A/I's in the pulsatile secretion rate $P_i(\cdot)$. Because the global constraint (equation A2) is a linear constraint, the resulting model is still Gaussian and the likelihood function is of the same basic form as that derived in Ref. 21, but with the mean function and covariances modified. The general asymptotic results of Refs. 21 and 51 are still applicable and were implemented in the estimation algorithms. Because total daily secretion and total daily basal secretion are now multiples of total daily pulsatile secretion, their SEs are multiples of the SE of total daily pulsatile secretion. Thus, in the constrained (C[24] and C[34]) model entries in Table 1, the SE for total daily secretion is now the sum of the SEs of total daily basal secretion and pulsatile secretion.

Wèthank Dr. William S. Evans (University of Virginia) for sharing reanalysis of the female LH data sets in this study, Dr. Thomas Mulligan (Virginia Commonwealth University) for allowing use of the LH infusion data, Paula P. Azimi for assistance in data presentation and graphics, and Patsy Craig for manuscript assembly.

This work was supported by the National Science Foundation Center for Biological Timing, the General Clinical Research Center (RR-00847), National Institutes of Health (NIH) Research.
REFERENCES


