Physiological control of pituitary hormone secretory-burst mass, frequency, and waveform: a statistical formulation and analysis

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1Department of Statistics, University of Virginia, Charlottesville, Virginia 22904; 2Department of Internal Medicine, Leiden University, Rapenburg 70, NA-2300 RA Leiden, The Netherlands; and 3Division of Endocrinology and Metabolism, Department of Internal Medicine, Mayo Medical School and Graduate School of Medicine, General Clinical Research Center, Mayo Clinic, Rochester, Minnesota 55905

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Keenan, Daniel M., Ferdinand Roelfsema, Nienke Biermasz, and Johannes D. Veldhuis. Physiological control of pituitary hormone secretory-burst mass, frequency, and waveform: a statistical formulation and analysis. Am J Physiol Regul Integr Comp Physiol 285: R664–R673, 2003.—The present study investigates the time-varying control of pituitary hormone secretion over the day and night (D/N). To this end, we implemented an analytical platform designed to reconstruct simultaneously 1) basal (nonpulsatile) secretion, 2) single or dual secretory-burst waveforms, 3) random effects on burst amplitude, 4) stochastic pulse-renewal properties, 5) biexponential elimination kinetics, and 6) experimental uncertainty. The statistical solution is conditioned on a priori pulse-onset times, which are estimated in the first stage. Primary data composed of thyrotropin (TSH) concentrations were monitored over 24 h in 27 healthy adults. According to statistical criteria, 21/27 profiles favored a dual compared with single secretory-burst waveform. An objectively defined waveform change point (D/N boundary) emerged at 2046 (±23 min), after which 1) the mass of TSH released per burst increases by 2.1-fold (P < 0.001), 2) TSH secretory-burst frequency rises by 1.2-fold (P < 0.001), 3) the latency to maximal TSH secretion within a burst decreases by 67% (P < 0.001), 4) variability in secretory-burst shape diminishes by 50% (P < 0.001), and 5) basal TSH secretion declines by 17% (P < 0.002). In contrast, the regularity of successive burst times and the slow-phase half-life are stable. In conclusion, nycthemeral mechanisms govern TSH secretory-burst mass, frequency, waveform, and variability but not evidently TSH elimination kinetics or the pulsing mechanism. Further studies will be required to assess the generality of the foregoing distinctive control mechanisms in other hypothalamic-pituitary axes.

METHODOLOGY

Methods

Clinical protocol. Healthy adults (N = 27) provided written voluntary, informed consent approved by the Institutional Review Board of the Leiden University Medical Center. After overnight adaptation to the inpatient study unit, subjects underwent repetitive blood sampling (2.0 ml every 10 min) for 24 h beginning at 0800. Fourteen men and thirteen women participated with respective mean ages of 46 and 41 yr (ranges: male 29–65 yr and female 32–50 yr).

Assay. TSH concentrations were quantitated in each serum sample (N = 145/24 h) in duplicate in a precise and sensitive immunofluorometric assay (Wallac Oy, Turku, Finland). The detection limit is 0.03 mU/l, the interassay coefficient of variation (CV) is 5%, and the intra-assay CV is 4.4% in the concentration (mU/l) range studied here (20).

Model of Diurnal TSH Secretion

Overall structure. The present study implements and adapts a recent Bayesian model of pulsatile neurohormone secretion, basal release, stochastic pulse times, flexible secretory-burst shape, random effects on burst mass, biexponential elimination kinetics, and combined experimental uncer-
Analytical reconstruction of dual bursts

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Fig. 1. A: model-based simultaneous dissection of a neurohormone concentration peak into underlying secretory-burst waveform (shape), discrete burst mass (integral of the secretory event), basal secretion rate, biexponential kinetics, and aggregate random effects of biological and experimental origin. Statistical solution is conditioned on a priori estimates of pulse-onset times (see Methods). B: flexible secretory-burst waveform shape encapsulated by the generalized Gamma probability distribution, defined by 3 beta parameters ($\beta_1$, $\beta_2$, and $\beta_3$). Discrete pulses are superimposed on time-invariant basal secretion ($\beta_0$). $S(t)$, secretion rate at time $t$.
discrete time sampling of
observational error, foregoing continuous processes, as distorted by superimposed
random variables, mental, independent, and identically distributed positive
ries monitored over 24 h in 1 individual.

Fig. 2. Comparison of single and dual secretory-burst model of neurohormone
secretion applied to 10-min serum thy-
rrotin (TSH) concentration time se-
secretory-burst model of neurohormone

\[ X(t) = (Ae^{-\gamma t} + (1 - A)e^{-\gamma T_0})X(0) \]
\[ + \int_0^t (Ae^{-\gamma(T-r)} + (1 - A)e^{-\gamma(T-r)}) \times Z(r)\,dr \]

where \( A \) is the (amplitude) proportion of rapid to total elimi-
nation, \( Z(t) \) denotes the aggregate secretion rate (see below), and \( X(0) \) gives the starting hormone concentration (7, 10). The observed hormone concentration profile, \( Y_i \), is then a discrete time sampling of \( N \) data points predicted by the foregoing continuous processes, as distorted by superimposed observational error, \( \epsilon_i \)

\[ Y_i = X(t_i) + \epsilon_i, i = 1, \ldots, N \] (5)

**Stochastic burst times.** Pulse times are viewed as arising by way of a stochastic process definable jointly by mean burst frequency and interburst-interval regularity (7, 11). This notion is represented by a two-parameter Weibull probability distribution. In the latter renewal process, successive pulse times, \( T_i \), emerge statistically as the partial sums of incremental, independent, and identically distributed positive random variables, \( S_i \)

\[ T_i = \sum_{j=1}^{i} S_i \] (6)

The classical Poisson counting process is a single-parameter renewal process identified by the rate parameter, \( \lambda \). In the Poisson model, random variables, \( S_i \), have an exponential distribution with a probabilistic average interval length (waiting time) of \( 1/\lambda \). However, the latter formulation does not accommodate possible biological dissociation between mean event frequency and interevent variability, inasmuch as the means \( \pm \) SD of the set of interpulse-interval lengths are equal definitionaly. Thus, in the Poisson model, the CV (\( \text{CV} = \text{mean/SD} \times 100\% \) of interburst waiting times is fixed at 100%. The latter diverges from estimated values of 15–40% for many hormones (4, 28, 30, 31). The Weibull probability density adds the parameter \( \gamma \), which uncouples mean burst frequency from statistical regularity of interpulse-interval lengths. In the Weibull construct, the conditional expectation for \( T^k \) given \( T_{k-1} \) is

\[ p(s|T^{k-1}) = \gamma \times \lambda^k (s - T^{k-1})^{-\gamma} e^{-\lambda s^{-\gamma}} \] (7)

where \( \lambda \) denotes the mean burst frequency (events observed/ unit time), \( \gamma \) is the regularity of interpulse-time delays, and \( s \) is a dummy variable on time (8, 11). The mean, variance, and CV of the Weibull renewal process are defined by

\[ \text{mean} = \frac{1}{\lambda^\gamma} \, \Gamma\left(1 + \frac{1}{\gamma}\right) \]
\[ \text{variance} = \frac{1}{\lambda^{2\gamma}} \times \left[ \Gamma\left(1 + \frac{2}{\gamma}\right) - \left[ \Gamma\left(1 + \frac{1}{\gamma}\right)\right]^2 \right] \] (8)

and \( CV = \frac{1}{\gamma + 1} - 1 \]

where \( \Gamma(\cdot) \) is the classical mathematical Gamma function (\( \Gamma \) and lowercase parameter \( \gamma \) are unrelated). Accordingly, in the Weibull density, the CV of interpulse (waiting) times depends only on \( \gamma \) (and not frequency \( \lambda \)). The Poisson process is a derivative function, wherein \( \gamma = 1 \). In the Weibull expansion, \( \gamma > 1 \) signif/ies more regular (less variable) interpulse-interval lengths.

**Data presentation.** Scatterplots are presented to highlight the dispersion of values in the cohort of 27 subjects. Values in the text are cited as means \( \pm \) SE, with median in parenthe-

![Image of Fig. 2](http://ajpregu.physiology.org/DownloadedFrom/)
ses. D/N parameter comparisons (dual- vs. single-burst waveform models) are made by a two-tailed, paired t-statistic. $P < 0.05$ was used to assign statistical significance.

RESULTS

Figure 2 illustrates application of single and dual secretory-burst waveform models to quantify secretion and elimination kinetics underlying 10-min observed 24-h serum TSH concentration profiles in one subject. The plots highlight estimated pulse-time locations (asterisks placed on $x$-axis), measured and model-projected TSH concentration profiles (top), reconstructed TSH secretion rates, the waveform change point (time; middle), and TSH secretory-burst shape (bottom).

Figure 3 depicts observed and analytically reconstructed serial TSH concentrations (top of each dip-tych) and calculated TSH secretion rates (bottom of each) under a postulated dual secretory-burst model in six normal adults. In the group of 27 subjects, the statistically defined change point (see Methods) occurred at clock time 2046 (±23 min) (2052).

Individual estimates of probabilistic TSH secretory-pulse frequency and D/N waveform-change point times are presented in Fig. 4. Daily TSH secretory burst number averaged 17.8 (±0.40) (18). The latter estimate (calculated a priori) is independent of subsequent choice of burst-waveform model (see Methods). Approximately 48% (±1.8%) (47%) of TSH pulses emerged...
within the 766±23 (772)-min daytime interval preceding the statistically identified change point. The 24-h normalized event frequency was 15 ± 0.44 (15) (based on a daytime projection) and 18 ± 0.57 (18) (nighttime projection) (P < 0.001).

As highlighted further in Fig. 4 (top), representation of 24-h TSH time series under a single- vs. dual-waveform model did not influence calculated biexponential TSH kinetics; namely, rapid-phase half-lives (in min) were 9.6 ± 1.6 (6.9) and 7.9 ± 0.60 (7.0), and slow-phase half-lives were 72 ± 7.3 (72) and 90 ± 11 (70) in the single- and dual-burst formulation, respectively. On statistical grounds, rapid-phase disappearance half-times are not determinable definitively for values less than the following: (ln 2)/sampling interval (here, 10 min; see Ref. 8). Therefore, the above rapid-phase kinetic estimates denote maximally realizable values.

Projected total daily TSH secretion rates (in mU·l⁻¹·24 h⁻¹) were comparable by model form; i.e., 60 ± 7.7 (50) and 56 ± 7.0 (50) in the single- and double-waveform constructs, respectively (Fig. 4, bottom). Pulsatile TSH secretion rates (summed daily pulse mass, in mU·l⁻¹·24 h⁻¹) were also independent of waveform representation; i.e., 40 ± 5.8 (26) (single-burst type) and 25 ± 5.2 (14) [two-burst type; P = not significant (NS)]. Basal secretion rates (in mU·l⁻¹·24 h⁻¹) were 21 ± 4.2 (16) (one-burst construct) and 25 ± 5.2 (14) (two-burst construct; P = NS), thus contributing approximately one-third of total TSH output.

Figure 5 (mode, mean, and SD) compares analytically estimated properties of TSH secretory bursts under the formalism of one or two distinct waveforms. In technical terminology, the ψ function defines the (unit-area normalized) time evolution of instantaneous secretion rates within any given burst (see Fig. 1B). The dual-waveform (2-ψ) formulation predicts significant D/N distinctions, namely, before and after the objective burst-shape change point (see Methods; Fig. 5B). Table 1 summarizes quantitative contrasts in the mode, mean, and SD of the predicted time latency (in min) to maximal secretion attained in the normalized secretory burst. An important inference is that the modal and mean times to peak TSH secretion are abbreviated markedly following (compared with before) the D/N waveform boundary; i.e., respective (D − N) difference values were 102 ± 15 (93) (modal time delay) and 112 ± 11 (107) min (mean time delay; both P < 0.001). Comparison of the interindividual variability (SD) of the mean time latency with maximal TSH
release revealed greater uniformity at night, i.e., difference of intersubject SDs between day and night: \(-43 \pm 14 (-17)\) min \((P < 0.001)\). The latter estimate signifies less between-person variability in TSH burst shape at night than during the day. The dual-waveform representations in Fig. 6 illustrate 1) the D/N distinction in burst-shape variability and 2) the nighttime shift toward more rapid TSH release within bursts. Predictions of a single-burst formulation are given by way of comparison. According to the AIC statistical penalty term (see Methods), a dual secretory-burst model represents diurnal and nocturnal

**Table 1. Daynight distinctions in analytically reconstructed measures of TSH secretory-burst waveform, shape, mass, and interpulse regularity**

<table>
<thead>
<tr>
<th>Measure of secretion</th>
<th>Model Type</th>
<th>Day</th>
<th>Night</th>
<th>Day</th>
<th>Night</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Single burst</td>
<td>Dual burst</td>
<td></td>
<td>Dual burst</td>
<td></td>
</tr>
<tr>
<td>Latency to maximum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mode</td>
<td>31 (\pm) 3.8 (30)</td>
<td>same</td>
<td>126 (\pm) 15 (123)</td>
<td>24 (\pm) 2.2* (22)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>108 (\pm) 12 (93)</td>
<td>same</td>
<td>164 (\pm) 13 (164)</td>
<td>55 (\pm) 6.1* (48)</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>110 (\pm) 17 (90)</td>
<td>same</td>
<td>85 (\pm) 16 (44)</td>
<td>45 (\pm) 8.5* (35)</td>
<td></td>
</tr>
<tr>
<td>Mass/burst</td>
<td>1.6 (\pm) 0.26 (1.2)</td>
<td>2.9 (\pm) 0.47 (2.3)</td>
<td>1.1 (\pm) 0.13 (1.0)</td>
<td>2.3 (\pm) 0.28* (2.1)</td>
<td></td>
</tr>
<tr>
<td>Pulsatile secretion</td>
<td>14 (\pm) 2.4 (11)</td>
<td>26 (\pm) 3.5* (19)</td>
<td>10 (\pm) 1.1 (10)</td>
<td>20 (\pm) 2.3* (17)</td>
<td></td>
</tr>
<tr>
<td>Weibull gamma</td>
<td>MI</td>
<td>MI</td>
<td>2.9 (\pm) 0.24 (2.4)</td>
<td>2.8 (\pm) 0.16* (2.6)</td>
<td></td>
</tr>
</tbody>
</table>

Numerical values are means \(\pm\) SE, with medians in parentheses \((N = 27\) subjects\). Latency to maximum, time to attain peak thyrotropin \((TSH)\) secretion after secretory-burst onset \((in\) min\); mass/burst, mU/l per secretory event; pulsatile secretion, mU \(\cdot\) l \(^{-1}\) \cdot\) 24 h \(^{-1}\) (normalized); Weibull gamma, regularity parameter \((higher\) values denote less variability\); MI, model independent \((prior\) estimation of pulse-onset times\). *\(P < 0.001\) and †\(P > 0.05\) vs. daytime value in same model.
TSH release more aptly (lower absolute AIC value) than a single-pulse construct in 21 of 27 individuals (Fig. 6, right). Figure 5 (mass and pulsatile) presents the calculated mass of TSH secreted per burst (in mU/l). Data are shown for the interval before vs. after the objective diurnal/nocturnal change point time in each subject. Cohort mean values are summarized in Table 1. On the basis of the statistical change point location inferred in the 2-H9274 model, we made D/N comparisons in both models. The latter revealed consistent nighttime amplification of burst mass, namely, by 1.8-fold (1 H9274) and 2.1-fold (2 H9274) (P < 0.001 vs. daytime). Pulsatile TSH secretion (in mU/l interval 1) rose commensurately, i.e., by 1.7-fold (1 burst) and 2.0-fold (2 bursts) (P < 0.001).

The regularity of the TSH pulse-renewal process (defined by analysis of the set of waiting times between consecutive bursts) was appraised statistically by γ of the Weibull renewal process (see Methods). Individual reconstructions of probability distributions and regularity (γ) of interpulse-interval lengths are shown in Fig. 7, and cohort mean values are given in Table 1. Statistical appraisal demonstrated stable interpulse-interval regularity (γ) over 24 h and elevated TSH pulse number (λ of Weibull process) after the waveform change point. Because the CV of interpulse-interval lengths (stochastic variability) is determined by γ independently of probabilistic mean event frequency (see Methods), these data indicate that the reciprocal property, regularity, of the TSH pulse-renewal process is stable nycthemerally.

DISCUSSION

The present composite formulation of deterministic and stochastic control of combined basal and pulsatile TSH secretion over 24 h introduces the concept of diurnal variation in secretory-burst waveform. Compared with a single-burst construct, dual-waveform representation of pulsatile TSH secretion was justified statistically in 21 of the 27 individuals. Implementation of the latter analytical construct disclosed that mechanisms driving the nocturnal elevation of plasma TSH concentrations include 2.1-fold amplification of TSH secretory-burst mass (in mU/l), 1.2-fold acceleration of probabilistic mean secretory-burst frequency (in events/h), and 67% abbreviation of the time latency (in min) to maximal TSH secretion within a burst. These day/night adaptations are specific, inasmuch as the quantifiable regularity of the stochastic pulse-renewal process and the in vivo elimination half-life of TSH did not vary over 24 h (Fig. 8). Whether the foregoing array of adaptive mechanisms is exclusive to physiological regulation of the TSH axis is not known. Generality of the current model form will be particularly relevant to explore in neuroendocrine systems that are marked by prominent diurnal and/or circadian rhythmicity, such as the corticotropic, somatotropic, lactotropic, and gonadotropic axes (4, 28, 30, 31).

The precise hypothalamo-pituitary mechanisms that orchestrate recurrent burstlike secretion of TSH in the day and night are unknown. Hypothalamic signals that impact thyrotropes include, nonexclusively, TRH (agonistic) and somatostatin and dopamine (antagonis-
tic) (1, 5, 16, 19, 24, 26, 39). Which, if any, of the foregoing effectors expressly determines the number and variability of discrete TSH secretory events is not established. In addition, the degree to which systemic feedback by triiodothyronine, thyroxine, and/or cortisol modulates the timing of discrete TSH secretory bursts is not clear. The present data indicate that, whatever the fundamental TSH pulse-generating process, relevant mechanisms accelerate TSH secretory-burst frequency at night without detectably altering intrinsic regularity of the stochastic pulse-renewal process.

Analytical reconstruction of TSH secretory-burst waveform (normalized rate of secretion over time) predicted significant abbreviation of the latency to maximal secretion at nighttime (Fig. 8). This novel adaptation could reflect nocturnally enhanced accumulation of readily releasable (exocytotically available) TSH stores, heightened thyrotrope sensitivity to agonistic inputs, and/or attenuated thyrotrope responsivity to antagonistic signals (1, 2, 14, 16, 19, 24–26, 37, 39, 40). For example, independent observations suggest that the hypothalamic outflow of somatostatin and dopamine may decrease over the nighttime interval, which decline may facilitate the sleep-related and late-day augmentation of GH and prolactin secretion, respectively (4, 22).

From a statistical vantage, a succession of discrete neurohormone secretory episodes can be viewed as random about a probabilistic mean waiting time (reciprocal of frequency) (7, 8). The dispersion of interevent delays measures the variability of the stochastic pulse-renewal process, here represented statistically by a Weibull distribution model. The latter formulation allows one to discriminate potentially separate biological control of burst frequency and interpulse regularity. For example, recent application of the Weibull analytical model unmasked paradoxically increased regularity of gonadotropin-releasing hormone (GnRH)/LH pulse regeneration in aging men and postmenopausal women compared with young counterparts (6, 11). Greater

Fig. 7. A: stochastic TSH pulse-renewal properties estimated via the 2-parameter Weibull probability distribution in relation to the day and night in 27 adults. The x-axis gives interburst-interval length (in min, reciprocal of frequency), and the y-axis gives the probability of observing that waiting time (A). B: relationship between probabilistic mean (24-h) TSH pulse frequency (λ, x-axis) and the statistical regularity of the set of randomly varying interburst intervals (γ, y-axis). TSH pulse frequency increases by 1.2-fold after the day/night change point, whereas interpulse-interval regularity does not change (Table 1).

Fig. 8. Overview of primary mechanisms of day/night control of TSH secretion. NS, not significant; , day; , night.
interpulse waiting-time regularity in the older male and female occurred in the face of elevated and unchanged daily LH pulse frequency, respectively. The precise neuroadaptive bases for physiological dissociation of stochastic interpulse-interval variability and pulse frequency in the thyrotropic and gonadotropic axes will be important to explore further.

Viewed mathematically, a stochastic pulse-renewal process denotes independently and identically distributed random, positive incremental waiting times. As one approach to validating this assumption in the case of TSH pulse regeneration, we applied the model-free and scale-invariant approximate entropy (ApEn) statistic. ApEn quantifies the order-dependent subpattern consistency (degree of process randomness) in numerical series (17, 35, 36). In a pulse-renewal process, the relative reproducibility of event recurrence times is definable by ApEn analysis of the observed sequence of successive interpulse-interval lengths. This application to TSH interburst waiting-time series revealed that the mean normalized ratio of ApEn of the original ordered series to ApEn of 1,000 randomly shuffled versions of the same series does not differ significantly from unity (not shown). The latter outcome is consistent with a presumptive stochastic pulse-renewal mechanism. ApEn analysis of sequential interburst-interval lengths in ACTH and LH time series has also predicted random pulse-timing properties in these systems (3, 7, 11, 33).

Perspectives

The precise nycthemeral determinants of secretory-burst mass, frequency, and waveform are not established. Depending on the neuroendocrine axis, relevant diurnally varying internal inputs could include central neural-, activity/rest-, and sleep/wake-related signals, whereas extrinsic factors often include fasting, food intake, external temperature, and light exposure. The present general analytical formulation should facilitate dissection of relevant underlying mechanisms mediating neuroendocrine adaptations. Further insights should be gained by extending statistical estimation procedures to include feedback and feedforward dose-responsive interface properties that control secretory-burst evolution, as foreshadowed in recent single-waveform analyses of the gonadotropic and corticotropic axes (7, 12).

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DISCLOSURES

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