Control of LH secretory-burst frequency and interpulse-interval regularity in women

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Keenan, Daniel M., William S. Evans, and Johannes D. Veldhuis. Control of LH secretory-burst frequency and interpulse-interval regularity in women. Am J Physiol Endocrinol Metab 285: E938–E948, 2003; 10.1152/ajpendo.00133.2003.—Hypothalamic neurons generate discrete bursts of gonadotropin-releasing hormone (GnRH) and thereby pulses of luteinizing hormone (LH) at randomly timed intervals centered on a probabilistic mean frequency. We tested the hypothesis that physiological mechanisms govern not only the number but also the stochastic dispersion of the GnRH/LH pulse-renewal process in humans; for example, in young women in the early (EF) and late (LF) follicular and midluteal (ML) phases of the menstrual cycle (n = 18) and in postmenopausal individuals (PM, n = 16). To this end, we quantify stochastic interpulse variability by way of the order-independent, two-parameter Weibull renewal process (Keenan DM and Veldhuis J. Am J Physiol Regul Integr Comp Physiol 281: R1917–R1924, 2001) and the sequence-specific, model-free approximate-entropy statistic (ApEn) (Pincus SM. Proc Natl Acad Sci USA 88: 2297–2301, 1991). Statistical testing unveiled 1) reduced probabilistic mean LH secretory-burst frequency (lower λ of the Weibull distribution) in ML compared with each of EF, LF, and PM (P < 0.001); 2) quantifiably more regular LH interburst-interval sets (elevated γ of the Weibull density) in PM than in each of EF, LF, and ML (P < 0.01); 3) uniquely prolonged latency to maximal LH secretion within individual secretory bursts in ML (P < 0.01); and 4) comparably mean random, sequential LH interburst-interval and mass values (normalized ApEn) among the distinct hormonal milieus. From these data, we postulate that sex steroids and age determine daily LH secretory-burst number, quantifiable pulse-renewal variability, and secretory-waveform evolution.

METHODS

Subjects. Study cohorts comprised 18 normal premenopausal women [mean age 26 (range 18–38 yr) and 16 PM subjects [mean age 58 (range 43–70 yr). Some volunteers had participated in an earlier independent analysis of LH production rates (14). Subjects provided voluntary, written, informed consent approved by the Institutional Review Board. Premenopausal volunteers were studied separately in the setting of normal gonadotrope responsivity. The basis for this inference includes the strong concordance between episodic GnRH and LH release in the rodent, lagamorph, ruminant, and primate (3, 18, 20, 27, 31, 36, 49), the consistent correspondence between arcuate nucleus electrophysiological correlates of GnRH outflow and individual pulses of LH in jugular venous blood in the monkey and rat (9, 46), and the rapid suppressibility of peripheral LH pulses by immunoneutralization of GnRH or pharmacological blockade of GnRH receptor (35, 37).

Synchronous GnRH neuronal firing and attendant LH secretory bursts recur at random intervals with no serial correlation of successive waiting times (1, 2, 12, 13). We recently highlighted the utility of quantifying the stochastic properties of the pulse-renewal process by the two-parameter Weibull probability distribution (10, 11). Unlike the derivative (1-parameter) Poisson process, the Weibull formulation statistically uncouples probabilistic mean event frequency from random variability in interevent delays. According to this analytic platform, there is physiological uncoupling of GnRH/LH pulse frequency (increased) and interburst waiting time variability (decreased) in the aging male with relative hypogonadism (15).

The present investigation tests the hypothesis that marked contrasts in the availability of estradiol and progesterone to enforce feedback on the hypothalamic-pituitary unit in menstruating premenopausal and estrogen-withdrawn postmenopausal women (PM) govern stochastic behavior of the implicit GnRH pulse generator and the reconstructed waveform of LH secretory bursts.

DIRECT APPRAISAL of hypothalamic gonadotropin-releasing hormone (GnRH) pulse-generator activity is not feasible in humans. However, the timing of discrete luteinizing hormone (LH) pulses in the peripheral circulation provides a surrogate marker of GnRH release in the setting of normal gonadotrope responsivity. The
the early follicular phase (EF; days 2–5 after onset of menses), late follicular phase (LF; 1–4 days before ovulation), and midluteal phase of the menstrual cycle (ML; days 5–8 after ovulation). The timing of ovulation was documented by serial transvaginal ultrasonography. The clinical diagnosis of menopause was affirmed by serum concentrations of FSH >50 IU/l and estradiol <20 pg/ml (72 pmol/l) and cessation of menstrual bleeding >12 mo earlier. Postmenopausal hormone replacement, if any, was discontinued at least 6 wk before the sampling procedure.

Clinical protocol. After overnight adaptation to the General Clinical Research Center, participants underwent repetitive (10-min) blood withdrawal over 24 h beginning at 0800. A forearm venous catheter was inserted at least 1 h before blood sampling. Subjects received breakfast, lunch, and dinner and were permitted to ambulate to the lavatory. Daytime naps, caffeinated beverages, and strenuous exercise were prohibited.

Hormone assays. LH concentrations were quantitated in duplicate by automated two-site monoclonal LH β-subunit-directed immunoradiometric assay (Nichols Laboratories, San Juan Capistrano, CA; see Ref. 44). Sera collected from an individual subject (n = 145 samples) were assayed together. Sensitivity according to the first International Reference Preparation is 0.2 IU/l and cross-reactivity with free α- or LH β-subunit <0.03%. Correlation with independent in vitro LH bioassay is r = +0.973 (45). The mean coefficient of variation (CV) within assays is 5.8% and between assays 8.3%.

Formulation of basal and pulsatile secretion convolved with elimination. Secretion and elimination parameters were estimated simultaneously from LH concentration time series, conditioned on estimated pulse onset times, as validated earlier on statistical and physiological grounds (Fig. 1 and Refs. 14, 17, and 42). Pulse onsets were identified by a previously described pulse detection method (14). The differential equation-based model incorporates pulse time estimation; combined pulsatile and basal release; biexponential hormone elimination kinetics; a flexible secretory-burst waveform; random effects on burst mass; and aggregate experimental uncertainty resulting from sample withdrawal, processing, and assay (12, 13, 15, 16). For a given set of pulse onset times (estimated in a first stage), the time-varying hormone concentration, \( X(t) \), is described by a set of differential equations (11), the solution of which is

\[
X(t) = \frac{a e^{-\alpha_1 t} + (1 - a)e^{-\alpha_2 t}}{1} X(0) + b_0 \left[ \frac{a}{\alpha_1} (1 - e^{-\alpha_1 t}) + \frac{1 - a}{\alpha_2} (1 - e^{-\alpha_2 t}) \right]
\]

“due to basal secretion”

where \( a \) is the relative proportion of rapid-to-total elimination amplitudes, \( \alpha_1 \) and \( \alpha_2 \) are the respective rate constants of the rapid and slow elimination phases, \( X(0) \) is the starting hormone concentration, \( b_0 \) is the basal secretion rate, \( t \) is time, and \( P(r)dr \) is the instantaneous pulsatile secretion rate over the infinitesimal time interval \( (r, r+dr) \) (see Ref. 11). Pulsatile secretion, \( P(r) \), is defined by the (summed) product of individual burst mass and the normalized secretory-event waveform

\[
P(r) = \sum_{r_i} M' \phi(r - r_i), \quad r > 0
\]

with \( M' = m_0 + m_1 (T_i - T_i^{-1}) + A' \)

and \( \phi(s) = s^{\beta_1 + 1} e^{-s^{\beta_2}}, \quad s \geq 0 \)

where \( M' \) denotes the mass of hormone released in the \( j \)th burst (per unit distribution volume); \( T_i \) is a pulse time; \( m_0 \) is minimal releasable hormone in the gland; \( m_1 \) is a proportionality constant of time-dependent mass accumulation over the interval \( T_i - T_i^{-1} \); \( A' \) indicates random variability in the mass of the \( j \)th secretory burst; and \( \phi(s) \) is the generalized three-parameter gamma family (normalized to integrate to 1).

The three-parameter waveform function \( \phi(s) \) creates flexibility of secretory-burst shape by allowing variable (β-specified) rates of upstroke, peak sharpness, and downstroke in the time-evolving release event (10). Specifically, allowable time asymmetry is achieved in the \( \phi \) function, as well as accurate approximation to the symmetric Gaussian waveform (39).

The total secretion rate is given by \( Z(\cdot) = b_0 + P(\cdot) \), that is, the arithmetic sum of basal and pulsatile release processes.

The observed LH concentration profile is a discrete time sampling of the analytic reconvoluted of the foregoing time-coupled continuous processes plus observational error (11, 14).

Model of pulse waiting times. We recently illustrated the utility of a Weibull renewal process model to describe random GnRH/LH and ACTH secretory-burst timing in the adult (10, 11, 15, 17). Mathematically, a renewal process \((T_k) \) results from the partial sums of incremental, independent, and identically distributed positive random variables, \( S_i \), with resultant \( T_k = \sum_{i=1}^{k} S_i \). A renewal process would encapsulate the expected intermittent output of an ensemble of randomly synchronized neurons, as inferred for GnRH neurons (7, 29, 34, 47, 48). The classical Poisson distribution defines a basic renewal process of probabilistic mean event frequency, \( \lambda \), in which the random variables, \( S_i \), have an exponential distribution with mean interval length, \( 1/\lambda \). The one-parameter Poisson model is limited in flexibility, since the mean and SD of interpulse-interval lengths are equal definitionally (15). The latter feature fixes the CV of waiting times at 100%, unlike inferred

\[
\frac{\gamma \times \lambda^3 (T_k^{\lambda - 1}) - \lambda^3 (T_k^{\lambda - 1})^\lambda e^{-\lambda^3 (T_k^{\lambda - 1})}}{\lambda^3 (T_k^{\lambda - 1}) - \lambda^3 (T_k^{\lambda - 1})^\lambda}
\]

physiological variability of 20–40% for interpulse delays (6, 35, 38).

In a Weibull renewal process, the conditional density for \( T_k \) given \( T_k^{-1} \) is given by

\[
P(s|T_k^{\lambda - 1} = \gamma \times \lambda^3 (s - T_k^{\lambda - 1})^\lambda e^{-\lambda^3 (s - T_k^{\lambda - 1})^\lambda}
\]

where \( \lambda \) denotes the probabilistic frequency (no. of events/unit time), and \( \gamma \) defines the statistical regularity of the set of
Fig. 1. Schematic overview of analytic reconstruction of primary secretion, elimination, and stochastic elements that drive time-varying neurohormone outflow. Li-gand secretion and kinetic properties are quantified simultaneously, conditioned on estimated pulse-onset times (see Methods).

interevent delays (10, 15). The Weibull distribution, wherein $\gamma > 1$, allows for greater regularity ($CV < 100\%$) than that of the derivative Poisson process defined by $\gamma = 1$ ($CV = 100\%$). The mean, variance, and CV of the Weibull probability density are

$$\text{Mean} = \frac{1}{\lambda} \times \Gamma\left(1 + \frac{1}{\gamma}\right)$$

$$\text{Variance} = \frac{1}{\lambda^2} \times \left\{ \frac{\Gamma\left(1 + \frac{2}{\gamma}\right)}{\Gamma\left(1 + \frac{1}{\gamma}\right)^2} - \left[ \frac{\Gamma\left(1 + \frac{1}{\gamma}\right)}{\Gamma\left(1 + \frac{1}{\gamma}\right)} \right]^2 \right\}$$

$$\text{CV} = \left[ \frac{\Gamma\left(1 + \frac{2}{\gamma}\right)}{\Gamma\left(1 + \frac{1}{\gamma}\right)} \right]^{1/2}$$

where $\Gamma(\cdot)$ is the classical mathematical gamma function (the latter is unrelated mathematically to the parameter $\gamma$). Accordingly, in the Weibull distribution, the CV of interpulse-interval lengths depends only on the parameter $\gamma$ (and not $\lambda$, frequency). Higher $\gamma$ denotes relatively greater regularity (lesser variability) of the set of random waiting times.

Approximate entropy analysis of sequence orderliness. The approximate entropy (ApEn) statistic is a model-free, scale-invariant, and order-sensitive regularity statistic that quantifies subpattern consistency in numerical sequences (24, 26, 41, 43). The normalized ApEn ratio was calculated for parameter pairs defined by vector length $m = 1$ and threshold $r = 0.2$ for data series of length $n \geq 30$ and $m = 1$ and $r = 1.0$ for $n < 30$ (25). The ApEn ratio is the quotient of random ApEn to observed ApEn, wherein random ApEn is estimated empirically here from 10,000 randomly shuffled versions (reordered without replacement) of the cognate series (41, 43). The ApEn ratio was applied to the succession of LH interpulse-interval lengths (min) and LH secretory-burst mass values (IU/l), normalized to preceding interpulse-interval length to decorrelate individual pulse mass from the $n_1$ term in Eq. 3.

Statistical analyses. The rate of secretory-burst evolution was compared by ANOVA followed by post hoc application of Tukey’s multiple-comparison criterion. The outcome measure was the time latency to maximal secretion within a release event. Moreover, generalized likelihood ratio tests for $\lambda$ and $\gamma$, based upon the Weibull model, were performed. End points were probabilistic frequency ($\lambda$) and interburst regularity ($\gamma$).

RESULTS

Figure 2 shows the serum LH concentration time series obtained by sampling peripheral blood every 10 min for 24 h in six premenopausal (two each in EF, LF, and ML) and six PM women. Plots depict observed and reconstructed (model-predicted) LH concentration profiles. The estimated pulse-onset times (see Methods) are shown in Fig. 2. Indicated $\gamma$ values quantify the regularity of interpulse waiting times, with higher $\gamma$ denoting less variability (lower CV of the Weibull distribution). Figure 3 presents analytically predicted LH secretory profiles in one LF and one PM individual and inferred waveforms of LH secretory bursts ($\phi$ function). The waveform denotes the time evolution of instantaneous LH secretion normalized to unit area to permit shape comparisons independently of mass (see Methods). Figure 4 summarizes statistical estimates of LH secretory-burst shape in all 34 subjects. ANOVA revealed that the rapid initial phase of LH secretion (time-to-peak LH release within any given burst) was significantly delayed in ML compared with that of each of EF, LF, and PM ($P < 0.01$).

To monitor frequency and stochastic regularity of the LH pulse-renewal process, we quantified burst frequency and waiting time variability according to the Weibull process model. Figure 5 illustrates analytic predictions for the two individuals identified in Fig. 4. Figure 6 presents interpulse-interval probability distributions in all volunteers. Generalized likelihood ratio tests, based on the Weibull model, revealed 1) a 40% lower modal LH pulse frequency ($\lambda$, 12 bursts/day) in ML than any of EF, LF, or PM (modal values 19–20; $P < 0.01$) and 2) an increased interburst interval regularity (elevated $\gamma$) in PM compared with each of EF, LF, and ML ($P < 0.001$). In particular, individual $\gamma$ estimates were 2.9–5.4 (absolute range) in PM com-
pared with cohort-specific modal values of 2.4–2.6 in the EF, LF, and ML. Figure 7 underscores the statistical and mechanistic independence of mean LH pulse frequency ($\lambda$) and interburst-interval regularity ($\gamma$) in each sex steroid milieu.

To quantify ad seriatim regularity of pulsatile LH release, we used the model-free, normalized ApEn ratio (see METHODS). Higher ApEn ratios (quotient of random to observed ApEn) define more orderly sequences (greater subpattern reproducibility). Figure 8 shows that ApEn ratios of successive LH interpulse-interval lengths and normalized secretory-burst mass values approach unity in the PM setting, thus approximating empirically mean random (based on ApEn recalculated on 10,000 shuffled versions of the cognate series). The foregoing estimates are consistent with stochastic renewal processes underlying the sequential timing and mass of secretory bursts (see DISCUSSION).

DISCUSSION

Simultaneous analytic reconstruction of the apparent in vivo waveform of asymmetric LH secretory bursts, probabilistic mean GnRH/LH secretory-event frequency, and stochastic interpulse-interval variability unmask novel regulatory contrasts in healthy young and older women. In particular, first, the inferred LH secretory-burst waveform (generalized gamma density) is uniquely skewed toward time-delayed maximal gonadotropin release in the ML com-
pared with EF, LF, and PM settings. Second, the probabilistic mean frequency of LH secretory bursts (\( \lambda \) of Weibull process) is singularly reduced in the ML compared with EF, LF, and PM contexts. Third, the quantifiable variability of the set of stochastically varying interpulse waiting times (\( \gamma \) of Weibull probability density) is distinctively diminished in the PM compared with EF, LF, and ML environments. And fourth, the (virtual) lack of quantifiable orderliness of successive LH secretory-burst mass and interpulse waiting time values (ApEn ratio) in each female cohort studied is consistent with predictions of statistical renewal processes defined by random (uncorrelated) event evolution. In ensemble, these findings point to mechanistically independent physiological control of the asymmetric waveform of LH secretory bursts, the probabilistic mean frequency of the GnRH/LH pulse-renewal process, and the random variability inherent in interburst time delays.

The ML phase is marked by a prolonged latency to attain maximal LH release in reconstructed secretory bursts. This stage of the menstrual cycle exhibits combined elevations of estrogen and progesterone concentrations. Because the time-to-peak LH secretion was not delayed comparably in the estrogen-enriched LF phase in young women, we hypothesize that progest-
erone-predominant negative feedback may be mechanistically relevant in determining LH secretory-burst waveform. Such determination could occur via direct or indirect actions exerted at the hypothalamic and/or the pituitary level. In contradistinction to the presently postulated control of the time evolution of LH release within any given discrete secretory burst, earlier laboratory and clinical data indicate that progesterone is able to repress mean hypothalamic GnRH pulse frequency in an estrogen-enriched environment, as also implied here (6, 28, 30, 39, 40).

In principle, the time evolution of an LH secretory burst is determined jointly by the instantaneous sensitivity and secretory capacity of gonadotrope cells to a fixed GnRH stimulus, the aggregate impact of effectual pituitary feedback signals, and the amount and time course of the incoming GnRH impulse. In relation to gonadotrope responsiveness in a progesterone-replete milieu, bolus intravenous injection of a single submaximally effective dose of GnRH evokes abundant LH release in the ML phase of the human menstrual cycle (32). In the absence of analytic reconstruction of implicit LH secretory-burst shape, the latter and other available experiments do not in fact examine GnRH-stimulated LH waveform development. Estrogen and progesterone can exert rapid membrane-level and delayed nuclear effects directly on gonadotrope cell signaling and LH β-subunit gene expression (4, 33). Whether such actions of sex steroids modulate the time evolution of the burst-like release of LH in vivo has not been elucidated.

The nature of the hypothalamic GnRH secretory-burst waveform in diverse sex steroid milieus and the precise impact of an individual GnRH secretory-burst...
time course on gonadotrope responses are not known. However, under in vitro perfusion and in vivo sampling conditions, the kinetics and the concentration of incident pulses of GnRH codetermine the amplitude, amount, and rate of consequent LH secretion (5, 8, 19). Accordingly, in principle, central-neural regulation of time-varying, burst-like GnRH outflow could also superintend LH pulse-waveform development in vivo. Definitive assessment of this fundamental issue will require direct and intensive sampling of GnRH secretory-burst evolution in hypothalamo-pituitary portal blood of the awake and unrestrained animal under experimentally defined sex steroid feedback.

The current formulation of stochastic LH pulse-renewal properties predicts commonality of probabilistic mean GnRH/LH pulse frequency in the EF, LF, and PM settings. This outcome diverges from some but not other earlier inferences (6). However, if corroborated in a larger cohort of healthy women, the present analyses would signify that estrogen availability per se is not a proximate determinant of the mean (nonpreovulatory) GnRH/LH pulse-renewal rate in humans. The latter proviso may be important in that the present data do not address the properties of random GnRH/LH secretory-burst timing during the preovulatory LH surge. Experimental inferences concerning the unique physiological transition that terminates the LF and introduces the luteal phase differ. For example, radiotelemetric monitoring of mediobasal hypothalamic multunit electrical-bursting activity (a putative physiological correlate of GnRH pulses) suggests that discrete GnRH event frequency decreases or does not

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**Fig. 5. Reconstruction of apparently random gonadotropin-releasing hormone (GnRH)/LH pulse-renewal properties.**

**A:** measured (10-min) LH concentrations over 24 h (continuous curves), estimated pulse-onset times (*), and model-estimated concentrations (dashed lines) in the 2 subjects (LF and PM) described in Fig. 2. **B:** stochastic (Weibull probability) distributions of interpulse-interval lengths (waiting times). $\lambda$ and $\gamma$ define the probabilistic frequency (no. of GnRH/LH secretory bursts evolving/24 h) and statistical regularity of randomly dispersed interburst-interval lengths, respectively. *Individual waiting times (min). $\gamma$ Values exceeding unity signify greater regularity (variability $<100\%$) than that of the Poisson process [wherein $\gamma = 1$ and the coefficient of variation of the process is definitionally 100%; see METHODS].
change during the preovulatory LH surge in the monkey and rat (22, 23). Other analyses based on systemic estimates of LH pulsatility and central monitoring of hypothalamo-pituitary portal-venous and cerebrospinal fluid GnRH pulsatility report amplification of GnRH pulse amplitude and variable acceleration of GnRH secretory-burst frequency during the ascending limb of the LH surge in the rat, monkey, and sheep (8, 21, 31, 36, 49). Such divergent insights would be consistent with species differences and more complex GnRH/LH regulatory mechanisms during the preovulatory interval (6).

Fig. 6. Interpulse-interval (Weibull) probability distributions in 6 EF (A), 6 LF (B), 6 ML (C), and 16 PM (D) women. The x-axis gives interpulse-interval length (min) and the y-axis the corresponding probability of observing any given waiting time. *Individual interburst time delays. The heavy dashed line denotes the Weibull density defined by median cohort parameters.
chastic renewal process wherein the sequence of pulse times is independent (see METHODS). A significant reduction of young adult variability in the GnRH/LH burst-renewal process in the PM female and aging male raises important mechanistic questions. A foremost query is whether unknown hypothalamic inter-neuronal adaptations and/or diminished sex steroid negative feedback in the older human drive paradoxically heightened regularity of the GnRH neuronal pulse-generating system.

In summary, the present clinical investigation delineates tripartite physiological control of LH secretory-burst waveform, probabilistic mean frequency of GnRH/LH events, and statistical regularity of the GnRH/LH pulse-renewal process in healthy women. Accordingly, in conjunction with independent studies in young and older men, we postulate that the sex steroid milieu and age jointly determine discrete facets of GnRH/LH signal generation in the female and male.

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DISCLOSURES

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