Cortisol Feedback State Governs Adrenocorticotropic Secretory-Burst
Shape, Frequency and Mass in a Dual-Waveform Construct:
time-of-day dependent regulation

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Abstract

Quantification of in vivo pituitary-hormone secretion requires simultaneous appraisal of implicit: (a) secretory-burst waveform, mass and stochastic pulse timing; (b) basal secretion; (c) biexponential elimination kinetics; and (d) random experimental error (Proc Natl Acad Sci 98: 4028-4033, 2001). The present study extends this analytical formalism to allow for time of day-dependent waveform adaptation (burst-shape change) at statistically determinable boundary times. Thereby, we test the hypothesis that diurnal mechanisms and glucocorticoid negative feedback jointly govern distinctive facets of the burst-like secretion of adrenocorticotropin (ACTH). To this end, we reanalyzed intensively (10-min) sampled 24-hr plasma ACTH concentration profiles collected previously under feedback-intact and drug-induced cortisol depletion in nine healthy adults. Akaike information criterion-based model comparison favored dual (rather than single) secretory-burst representation of 24-hr ACTH release in both the intact and low-cortisol setting in 8 of 9 subjects. Under feedback-intact conditions, analytically predicted waveform changepoints (median clock times 0611 h and 1739 h) flanked an interval of elevated ACTH secretory-burst mass (P < 10^{-3}). Experimental hypocortisolemia did not alter day/night boundaries, but: (a) stimulated day ACTH secretory-burst mass (P < 10^{-3}); (b) accelerated day ACTH secretory-burst frequency (P < 10^{-3}); and (c) forced skewness of day ACTH secretory bursts toward more rapid initial release (P < 0.05). In contrast, the basal ACTH secretion rate and regularity of interpulse-interval lengths were invariant of day/night segmentation and cortisol availability. In conclusion, unknown diurnal factors and systemic cortisol concentrations codetermine ACTH secretory-burst waveform, frequency and mass, whereas neither mechanism regulates basal ACTH release or regularity of the burst-renewal process.
Introduction

The stress-responsive corticotropic axis comprises (minimally) circadian inputs, hypothalamic peptides, pituitary corticotropes, sympathetic pathways, the adrenal gland and glucocorticoid receptors (7;23). Central regulation unfolds via paraventricular nuclear release of corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP) into hypophyseal portal blood in discrete bursts (1;30). Intermittent neuronal outflow of CRH and AVP putatively constitutes the primary pulse-renewal process. These secretagogues stimulate exocytotic release and de novo synthesis of adrenocorticotropic hormone (ACTH) by the anterior pituitary gland (1;5;9;24;25;32;40). Secreted ACTH drives the production of aldosterone and glucocorticoids by the adrenal zona glomerulosa and fasciculata (9). Glucocorticoids in turn inhibit hypothalamic secretion of CRH and AVP and directly repress corticotrope secretion of ACTH (2;4;8;10;22;29;30;41). According to this ensemble perspective, we postulated that time of day and glucocorticoid-dependent negative feedback conjointly control ACTH secretory-burst number, timing, mass and waveform and/or basal ACTH release, but not plasma ACTH elimination (23;30). The present analyses test this regulatory postulate by analytical reconstruction of ACTH secretion and elimination rates under feedback-intact and feedback-depleted conditions in the healthy human.

Methods

Clinical Protocol

Conventional pulse analysis of the ACTH time series was reported earlier (38). The present analytical platform and hypotheses do not overlap in any
manner. Briefly, nine volunteers participated in the study (five men and four women). Each participant provided written informed consent approved by the primary Institutional Review Board [Acknowledgements (38)]. Median ages were 39 (range 24 to 48) yr in women and 51 (38 to 62) yr in men, and body mass indices (BMI) 27 (23 to 31) kg/m² and 25 (21 to 29) kg/m², respectively. Participants maintained conventional work and sleeping patterns, and reported no recent (within 10 days) transmeridian travel, weight loss or gain, intercurrent psychosocial stress, prescription medication use, substance abuse, neuropsychiatric illness or systemic disease. A complete medical history, physical examination, structured psychiatric interview, and screening tests of hematological, renal, hepatic, metabolic and endocrine function were normal. No volunteer had been exposed to glucocorticoids within the preceding 3 months.

Volunteers were admitted to the General Clinical Research Center at 1900 h. A catheter was placed in a forearm vein at 2000 h. Beginning at midnight, subjects were given oral capsules of placebo (day 1 of 2) or metyrapone (day 2) with milk and crackers every 2 hr for 24 hr. The dosing schedule of metyrapone was 1000 mg orally every 2 hr for six doses, followed by 500 mg every 2 hr for six additional doses. Meals were provided at 0730, 1200 and 1800 h. Blood samples (1.6 ml) were withdrawn at 10-min intervals from midnight onward for 48 hr. Specimens were collected in chilled EDTA-containing tubes, centrifuged at 4C to separate plasma, and frozen at -70C. Total blood loss was 490 ml. Volunteers were compensated for the time spent in participation.
Hormone Assays

ACTH was quantitated in duplicate by high-sensitivity robotics-automated immunoradiometric assay with median within- and between-assay coefficients of variation of 5.2 and 6.1%, respectively (13;38). Cortisol was assayed by solid-phase RIA (37).

Analytical Platform

(a) Overview

The core model is a statistically validated construct of combined feedback/feedforward control of the corticotropic axis (17). The present analyses develop and apply a dual secretory-burst waveform formulation of ACTH pulses in the feedback intact (control) and feedback-depleted (metyrapone) contexts. The basic construct (highlighted below) incorporates stochastic pulse timing (two-parameter renewal process); combined pulsatile and basal modes of secretion; subject-specific biexponential elimination kinetics; a flexible (three-parameter) secretory-burst waveform; random-effects on sequential burst mass; and, experimental uncertainty due to sample withdrawal, processing and assay (18;21). In addition, we introduce a statistically identifiable pair of changepoints arising within any given 24-hr ACTH time series, which demarcate an interval of analytically distinguishable secretory-burst waveform: Figure 1A.

(b) Secretion and elimination functions

We earlier showed that time-varying hormone concentrations, X(t), are described by a set of coupled differential equations describing all three of basal release, pulsatile secretion and biexponential elimination, as follows:
\( X(t) = (Ae^{-\alpha_1 t} + (1 - A)e^{-\alpha_2 t})X(0) + \beta_0 \times \left( \frac{A}{\alpha_1} (1 - e^{-\alpha_1 t}) + \frac{(1-A)}{\alpha_2} (1 - e^{-\alpha_2 t}) \right) + \int_{0}^{t} (Ae^{-\alpha_1(t-r)} + (1 - A)e^{-\alpha_2(t-r)}) \times P(r)dr \)

\approx \beta_0 \times \left( \frac{A}{\alpha_1} (1 - e^{-\alpha_1 t}) + \frac{(1-A)}{\alpha_2} (1 - e^{-\alpha_2 t}) \right) + \int_{0}^{t} (Ae^{-\alpha_1(t-r)} + (1 - A)e^{-\alpha_2(t-r)}) \times P(r)dr

"due to basal release" + "due to pulsatile secretion"

where \( A \) is the proportion of rapid to total elimination, \( \alpha_1 \) and \( \alpha_2 \) are the respective rate constants of the rapid and slow elimination phases, \( X(0) \) the starting hormone concentration, \( \beta_0 \) the basal secretion rate, \( t \) time and \( P(r)dr \) the instantaneous pulsatile secretion rate over the infinitesimal time interval \( (r, r + dr) \) \( (17;18) \).

The pulsatile secretion function is defined by:

\[ P(r) = \sum_{r \leq T} M^j \psi(r - T^j), \ r \geq 0 \]

with \( M^j = \eta_0 + \eta_1 \times (T^j - T^{j-1}) + A^j \)

and \( \psi(s) \propto s^{\beta_1 \beta_2 - 1} e^{-(s/\beta_2)^{\beta_3}}, \ s \geq 0 \)

where \( \psi(s) \) denotes the generalized (three-parameter) Gamma probability distribution normalized to integrate to unity; \( M^j \) the mass of hormone released in the \( j^{th} \) burst per unit distribution volume; \( \eta_0 \) basal hormone availability for release by the secretory gland; \( \eta_1 \) a rate constant of hormone accumulation over the time interval, \( T^j - T^{j-1} \); and, \( A^j \) random effects on the mass of the \( j^{th} \) secretory burst.

The three parameters of the \( \psi \) function ensure flexibility of secretory-burst shape by allowing for a broad range in the upstroke, peakedness and downstroke of the secretory-burst waveform. Asymmetric as well as symmetric (e.g., Gaussian)
representations of secretory events are well represented by the three-parameter Gamma density.

The total secretion rate is given by \( Z(\cdot) = \beta_0 + P(\cdot) \), which denotes the sum of basal and pulsatile release.

(c) Dual-waveform model of burst-like pituitary-hormone secretion

We test the hypothesis that there are two finite and statistically determinable transition times (changepoints) within any given 24-hr time series, which bound the occurrence of an analytically distinguishable (second) waveform of secretory bursts. Objectively, the changepoint demarcates the appearance or the disappearance of statistically independent putative day, \( \psi^{(D)} \), and night, \( \psi^{(N)} \), waveform functions defined by corresponding parameters, \( (\beta_1^{(D)}, \beta_2^{(D)}, \beta_3^{(D)}) \) and \( (\beta_1^{(N)}, \beta_2^{(N)}, \beta_3^{(N)}) \). Mathematically, the resultant parameter set for maximum-likelihood estimation (MLE) includes five additional parameters (two changepoints and three parameters of the new secretory-burst waveform). According to this construction, there may or may not be a statistical requirement for representation of dual secretory-burst waveforms. The distinction is made on statistical grounds, wherein the one and two-waveform model outcomes for each data set are compared via the Akaike Information Criterion (AIC). Specifically, suppose that there are two models, the first parameterized by \( p \) parameters, and the second, a larger model that contains the first and is parameterized by \( p + m \) parameters. The AIC criterion states that the second model is appropriate if twice the number of additional parameters, \( 2m \), is less than twice the log value of the likelihood ratio test of the first to the second model. The factor of two affords ease of Chi-square
calculation. The AIC measure penalizes enhancement of the regression fit (sum of squares of residuals) achieved solely by adding $m$ new parameters, such that a lower value favors a given model based upon a principle of assumed statistical parsimony.

The observed ACTH concentration profile is a discrete time sampling of the foregoing underlying continuous processes plus observational error (18;19).

(d) Model of pulse-waiting times

We recently illustrated utility of a statistical renewal process to describe randomly emergent LH pulse times (20). The model is also applicable to the presently observed ACTH pulse times. Mathematically, a renewal process ($T^k$) results from the partial sums of incremental, independent and identically distributed positive random variables, $S_i$, with resultant $T^k = \sum_{i=1}^{k} S_i$. In the present analysis, successive $S_i$ values denote consecutive interpulse waiting times (min). This model structure would reasonably represent intermittent output of an ensemble of randomly synchronized neurons (6). The one-parameter Poisson distribution defines a basic renewal process, in which interevent intervals, $S_i$, have an exponential distribution. In the Poisson process, the mean and standard deviation (SD) of the set of interpulse-interval lengths are equal definitionally. The latter feature fixes the coefficient of variation (CV) of waiting times at 100%, which differs significantly from inferred physiological interburst-time variability of 20 to 40% for LH, GH, FSH, prolactin, insulin, parathyroid hormone and ACTH (11;17;31;33;34;36).
To allow flexibility of the interpulse-interval CV among hormones and individual subjects, we utilize the two-parameter Weibull probability distribution. In a Weibull renewal process, the conditional probability density for $T^{k}$ given $T^{k-1}$ is given by

$$P(s \mid T^{k-1}) = \gamma \times \lambda^\gamma (s - T^{k-1})^{\gamma - 1} e^{-\lambda^\gamma (s - T^{k-1})^\gamma}$$

where $\lambda$ designates the probabilistic mean frequency (expected number of events/unit time), and $\gamma$ the regularity of the set of interpulse waiting times. In the Weibull density, $\gamma > 1$ denotes greater regularity (lesser variability, CV < 100%) than that of the Poisson model (wherein $\gamma = 1$ by construction). The mean, variance and CV of the Weibull distribution are:

\[
\text{MEAN} = \frac{1}{\lambda} \times \Gamma\left(1 + \frac{1}{\gamma}\right)
\]

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

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

where $\Gamma(\cdot)$ is the classical algebraic Gamma function (the latter is unrelated mathematically to the parameter $\gamma$). Accordingly, in the Weibull distribution the CV of random interpulse-interval lengths depends expressly on $\gamma$ (and not $\lambda$, frequency), and higher $\gamma$ signifies greater regularity (a lesser CV).

**Statistical Analysis**

Scatterplots are used to illustrate between-subject dispersion of certain measures. Data are given as the mean ± SEM (median) in the text and tables. Likelihood-based tests were used to contrast secretion and kinetic parameters.
during placebo and metyrapone administration. Significance was construed for P < 0.05.

**Results**

Metyrapone-induced inhibition of adrenal glucocorticoid synthesis reduced the mean (24-hr) serum cortisol concentration to < 5.2 µg/dL (versus normal diurnal sample range, 10 to 28 µg/dL). Initial analyses tested the analytical relevance of dual (two ψi) versus single (one ψi) ACTH secretory-burst waveform reconstruction of 24-hr episodic ACTH secretion. Application of the AIC penalty term [Methods] revealed statistically preferred representation (AIC difference > 0) of 24-hr ACTH release by a two-ψi versus one-ψi formulation in 8 of 9 subjects in both the intact and low-cortisol feedback settings. According to the dual-burst model, analytically predicted changepoints (clock times [h] ± min [median]) were 0638 h ± 23 [0611 h] and 1833 h ± 61 [1739 h] in the feedback-intact setting and 0659 h ± 25 [0654] and 1708 h ± 33 [1719 h] in the hypocortisolemic context:

**Figure 2.** For discussion purposes, we designate the foregoing inclusive time windows as day, and the combined excluded (two flanking) intervals as night.

**Figure 3A** illustrates observed and analytically predicted 24-hr plasma ACTH concentration time series, corresponding model-projected ACTH secretion rates, and statistically estimated waveform changepoint times in two individuals. **Figure 3B** presents metyrapone-stimulated ACTH release profiles in the same two subjects (and repeats the rescaled control data for comparison). **Figure 4A** and **4B** depict representative analytical reconstruction of ACTH secretory bursts in one
individual under the two models of waveform evolution. The dual-burst formulation shows prominent waveform segmentation by the day and night.

**Figure 5A** summarizes the primary parameters of secretion and elimination estimated statistically by way of the one and two ACTH-waveform constructs. Both representations are conditioned on the same set of *a priori* estimates of pulse-onset times. Statistical comparison of the paired (N=9) data disclosed the following salient effects of hypocortisolemia (metyrapone) compared with eucortisolemia (control) on 24-hr ACTH dynamics in both models: (i) significantly elevated daily (total) ACTH secretion, attributable principally to augmentation of the calculated mass of ACTH secreted per burst (MPB, µg/L) and, to a lesser extent, acceleration of ACTH secretory-burst frequency (lambda of Weibull distribution, bursts/24 hr); and (ii) no change in the slow-phase ACTH half-life (min), basal ACTH secretion rate (µg/L/24 hr) and interpulse-interval regularity (gamma of Weibull probability density). Accordingly, statistical model choice does not bias the prediction of key parameters of 24-hr ACTH secretion or elimination.

The dual-waveform construct unveiled significant day-night contrasts in: (i) waveform shape (below); and (ii) ACTH secretory-burst mass (elevated in the day by 1.6-fold, P = 0.02): **Table 1**. In the eucortisolemic setting, there were no detectable diurnal adaptations in basal ACTH secretion, ACTH secretory burst frequency or pulse-renewal regularity: **Figure 5B**. In the hypocortisolemic milieu, there was 2.4-fold greater (24-hr normalized) pulsatile ACTH production in the day than night (P < 0.005). The daytime increase was accounted for specifically by: (a) 2.0-fold amplification of the mass of ACTH released per burst (P < 0.005); and (b) 1.27-fold augmentation of ACTH secretory-event frequency (lambda) (P < 0.001).
There were no day-night differences in basal ACTH secretion or interburst-interval regularity (gamma) [Figure 5B, Table 1]. In addition, the calculated slow-phase half-life (min) of plasma ACTH elimination was unaffected by glucocorticoid withdrawal; viz., 14 ± 0.94 (14) [control] versus 15 ± 3.1 (11) [metyrapone].

Figures 6A and 6B depict analytically predicted ACTH secretory-burst waveforms in each of 9 subjects according to the dual and single-burst models, along with intraindividual AIC differences to compare model performance. Data are given in the intact (control) and low (metyrapone) feedback state. Based on the 3-parameter generalized Gamma density representation of underlying secretory bursts (normalized to integrate to unit), the time evolution (shape) of the burst is viewed independently of mass (Methods). Thereby, we compare estimated burst kinetics. Table 2 summarizes cohort estimates of quantile time latencies (min) to secrete a given percentage of ACTH within a burst (here 15%, 35%, median [50%], 75% and 90%). The salient distinction statistically is a daytime-restricted abbreviation of the time required to release 35 to 75% of total ACTH in the low glucocorticoid-feedback environment (P < 0.05).

The Weibull density was used to permit simultaneous estimation of the frequency of secretory bursts (lambda) and the regularity of the putative stochastic pulse-renewal process (gamma): Table 1. Frequency is represented as the inverse of interburst-interval length. Interval lengths estimated in any given subject over 24 hr are envisioned analytically as a probability distribution: Figure 7A. Day versus night segmentation did not influence mean or median interpulse-interval length (left panels). However, metyrapone exposure reduced daytime interval lengths (P < 0.001; Figure 7A, right panels and Table 1). Figure 7B highlights the
relationship between predicted ACTH pulse frequency and stochastic interpulse waiting-time regularity in the glucocorticoid-sufficient and cortisol-depleted settings.

**Discussion**

The present investigation identifies time of day-dependent mechanistic adaptations of the hypothalamo-corticotrope unit that are specific to the cortisol feedback-intact and feedback-restricted settings. The day-night distinction required statistical segmentation of ACTH secretory-burst shape evolution over 24 hr into two analytically projected waveforms demarcated by determinable changepoints (boundaries in time). Thereby, we show that reduced cortisol availability during the statistically defined day amplifies ACTH secretory-burst mass (by 9.6-fold), accelerates mean ACTH pulse frequency (by 1.27-fold), and skews ACTH secretory-burst shape toward more rapid release (median latency decrement 31 min). In contradistinction, according to the current analytical formulation, neither time-of-day nor cortisol concentrations control basal (nonpulsatile) ACTH secretion, the plasma ACTH half-life or the stochastic regularity of ACTH interburst intervals.

An important prediction in the current work is that episodic ACTH secretion is nonuniform in waveform evolution over 24 hr. This insight is supported by formal intraindividual statistical comparisons that favor a dual over a single secretory-burst waveform to represent time-varying ACTH release in 8 of 9 subjects, as assessed separately in the glucocorticoid feedback-intact and feedback-restricted setting [Figure 6]. The analytically defined onset/offset time boundaries (median changepoints) of the distinguishable waveforms were comparable in
eucortisolemia (0611 h and 1739 h) and experimental hypocortisolemia (0654 h and 1719 h). This outcome implies that metyrapone administration (at midnight) has no discernible effect on the objectively forecast timing of the ACTH secretory-burst waveform transition. In fact, the precise neuroregulatory mechanisms driving inferred ACTH waveform evolution over 24 hr are not immediately evident, but might involve circadian signals.

The intraindividual day-night difference in ACTH secretory-burst frequency (normalized to pulses/24 hr) rose from a nonsignificant median value of -0.83 in the cortisol feedback-intact setting to +12.3 during reduced glucocorticoid feedback ($P < 0.001$). Daytime-restricted acceleration of ACTH pulse frequency in the low cortisol milieu would presumptively signify corresponding time of day-specific augmentation of the number, amplitude and/or signaling efficacy of hypothalamic CRH and/or AVP secretory bursts driving responsive corticotropes: **Figure 8.** We postulate specifically that: (a) frequency enhancement in the day may denote an increased number of CRH and/or AVP pulses; (b) amplitude amplification of ACTH secretory bursts would plausibly reflect diurnal augmentation of the mass of CRH and/or AVP pulses associated with elevated CRH and AVP gene expression in the circadian day (3;6); (c) the combined daytime rise in ACTH secretory-burst mass and number may mirror disinhibition of direct pituitary suppression by lower cortisol concentrations, inasmuch as enhanced CRH/AVP feedforward efficacy or potency would predictably facilitate emergence of more readily detectable high-amplitude ACTH pulses; and (d) hypocortisolemia may heighten the (temporal) concordance between discrete CRH and AVP release episodes, thereby accentuating secretagogue synergy (2;5;8;14;15;22;24;25;27-
The only experimental assessment of simultaneous release of all three of hypothalamo-pituitary CRH, AVP and ACTH under hypocortisolemia based on direct sampling of the cavernous sinus in the unanesthetized unrestrained horse for 4 hr was not definitive on this point (2). The latter analysis was made difficult by a limited (30-min) time window of baseline monitoring prior to intravenous infusion of metyrapone.

The low cortisol-feedback state stimulated 24-hr (total) ACTH secretory-burst mass markedly; viz., by 8.8 and 9.6-fold according to the single-burst and dual-waveform models, respectively. According to the foregoing data, the type of waveform reconstruction used does not confound this fundamental outcome. Analytically, secretory-burst mass denotes the amount (µg) of ACTH secreted per unit distribution volume (L) within a delimited release episode. Comparison of ACTH release in the day and night segments in the dual ACTH secretory-burst model identified 1.6-fold and 2.0-fold greater day than night values in the intact and low cortisol-feedback milieus, respectively. Thus, the overall effect of day and low cortisol feedback on ACTH secretory burst mass was multiplicative (17-fold over night and normal-cortisol feedback).

Glucocorticoid-feedback withdrawal did not elevate basal (time-invariant) ACTH secretion significantly. This inference assumes technically valid discrimination of statistically intercorrelated measures of hormone secretion and elimination (17;18;21). The present result differs from that of an earlier multiparameter deconvolution procedure applied to the same plasma ACTH concentration time series (35;38). Computer-assisted simulations indicate that the original multiparameter approach is hampered by strong (sixfold) covariance
among estimates of basal secretion, endogenous (monoexponential) half-life, and secretory-burst number, timing, amplitude and duration (39). The foregoing issues motivated the current analytical strategy of first-stage modeling and estimation of pulse-onset times, followed by second-stage simultaneous estimation of secretion and biexponential elimination parameters by a maximum-likelihood methodology (17-19). Robustness of the present computation of basal ACTH secretion is evident practically in the consistency of this measure in the day and night and independently of choice of secretory-burst model. For example, in the dual and single-burst constructs, percentage basal (of total) ACTH secretion averaged 27% and 33% during eucortisolemia and 6.4% and 6.5% during hypocortisolemia, respectively. The percentage decrease is a predictable consequence of comparable absolute basal and markedly elevated pulsatile ACTH secretion in response to reduced glucocorticoid feedback.

Glucocorticoid availability and time-of-day jointly determined estimates of ACTH secretory-burst waveform (shape, as defined by the time evolution of instantaneous secretion rates). Specifically, low cortisol concentrations evoked ACTH secretory events that were significantly skewed toward more rapid initial ACTH release. This feedback adaptation emerged exclusively in the day interval (Table 2). In the generalized Gamma-density probabilistic model applied here, the (unit area-normalized) secretory-burst waveform may be compared between interventions independently of a change in total burst mass (Methods). Three parameters of waveform shape allow for unequal peakedness (sharpness of the maximum) and rates of upstroke and downstroke within the predicted secretory burst (Figure 1), thereby obviating the symmetry constraint inherent in a two-
parameter Gaussian model (17;18;35). Secretory-burst asymmetry is inferable indirectly from time profiles of CRH and AVP-stimulated ACTH release monitored during *in vitro* perifusion of corticotropes and directly *in vivo* via cavernous-sinus sampling of ACTH secretion (8;26;27;40). From a mechanistic vantage, a plausible postulate is that daytime hypocortisolemia evokes more rapid initial ACTH secretion by potentiating corticotrope exocytosis of prestored hormone. This hypothesis would accommodate putatively greater nighttime accumulation of releasable ACTH stores under low glucocorticoid feedback followed by enhanced daytime stimulation of ACTH release by CRH and/or AVP (2;4;8;12;24;25;30;32;40;41). Physiological control of pituitary-hormone secretory-burst shape is also evident in relation to pulsatile luteinizing hormone (LH) release assessed at different stages of the normal menstrual cycle (16).

To quantify stochastic regularity of the putative CRH and AVP pulse-generating mechanism, we formulated ACTH intersecretory-burst intervals as the output of a classical Weibull renewal process (17). The latter probability model permits combined estimation of mean frequency (lambda) and interburst waiting-time regularity (gamma) (33;34). For example, in a general Weibull formulation, independently of lambda, gamma > 1 denotes greater interpulse-interval regularity (CV < 100%) than that defined by the derivative Poisson model (wherein gamma = 1 definitionally, forcing a fixed interpulse-interval CV of 100%) (17;18;20). The present analysis indicates that the inferred hypothalamic CRH/AVP burst-renewal process is more regular than that predicted by a classical Poisson construct, inasmuch as median gamma values ranged from 2.4 to 3.0 (Table 1). Statistical regularity of interpulse-interval lengths did not differ by way of day/night.
segmentation, cortisol feedback state or mean ACTH burst frequency. Although few comparable data are available as yet in other neuroendocrine axes, regularity of the GnRH/LH pulse-renewal process is significantly higher (denoting less interevent waiting-time variability) in healthy older men and postmenopausal women than gender-matched young subjects (16;20).

**Perspectives**

From an integrative perspective, the present investigation highlights the utility of linking empirical data and statistical developments to probe more complex regulatory physiology. In particular, experimental findings foster relevant revisions of feedback concepts, and evolving statistical platforms promote new insights into physiological mechanisms. The foregoing notions are illustrated in queries raised by the current combined implementation of a feedback intervention and a new analytical methodology to appraise corticotropin secretion. For example, emergent questions include the precise nature of neurophysiological mechanisms that determine probabilistic hypothalamo-pituitary secretory-burst frequency and stochastic regularity; and, conversely, the appropriate casting of informative statistical models to embody time-varying multisignal control of day/night and feedback-adaptive secretory-burst waveform.
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Legends

Figure 1. Schema of simultaneous analysis of hormone secretion and elimination conditioned statistically on prior estimates of pulse-onset times assuming a single versus dual secretory-burst waveform (shape). Lower panels depict [left to right]: waveform distinction; stochastically varying burst mass; statistically identified changepoints (vertical interrupted lines, time boundaries of waveform adaptations); biexponential elimination kinetics; and random effects (experimental uncertainty).

Figure 2. Lack of cortisol-dependent feedback control of statistically predicted ACTH secretory-burst changepoint times. Numerical values are the mean (± SEM) clock times (h ± min) for eucortisolemia (control) and hypocortisolemia (metyrapone).

Figure 3. Plasma ACTH concentration time series (top) [observed, continuous lines; predicted, interrupted lines] and analytically reconstructed ACTH secretion rates (bottom) based on a dual secretory-burst waveform construct. Time series are illustrated in two normal adults at baseline [control, Panel A] and during hypocortisolemia [metyrapone, Panel B]. Subjects underwent peripheral blood sampling every 10 min for 24 hr. Objectively defined (statistically parameterized) changepoints (paired bold-face open circles) designate the time boundaries of night and day-delimited ACTH secretory-burst waveform.

Figure 4. Reconstructed ACTH secretory-burst waveform under intact cortisol feedback (control, Panel A) and reduced cortisol feedback (metyrapone, Panel B). The two top panels illustrate measured (continuous curve) and predicted (reconstructed, interrupted curve) 24-hr ACTH concentration time series in a single-burst [left] and dual-waveform [right] model. The middle panels depict
corresponding analytically predicted ACTH secretory rates. The bottom panels show model-, intervention-, subject- and time of day-dependent predicted secretory-burst waveforms. The waveform is defined as the time-evolution of instantaneous ACTH secretion rates within a discrete release episode. Burst shape is represented algebraically by a generalized Gamma probability distribution, normalized to unit area to facilitate shape distinctions independently of mass secreted (Methods). Statistical contrasts are given in the text and Tables.

**Figure 5.** Impact of eucortisolemia (control, top) and low glucocorticoid feedback (metyrapone, bottom) on regulated facets of ACTH secretion in the night (Nt) and day according to a dual secretory-burst construction [Methods]. Panel A, left to right: total daily, basal (nonpulsatile) and pulsatile ACTH secretion and mean mass of ACTH secreted/burst. Panel B: overall ACTH pulse frequency (lambda), night/day (N/D) lengths (or segment durations), 24 hr-normalized ACTH burst frequency for the night and day segments, and interburst-interval regularity (gamma) in the night and day. Data are depicted for individual subjects. Interrupted lines connect median values of the cohort [N=9]. Statistical comparisons are summarized in the text and Tables 1 and 2.

**Figure 6.** Comparisons of night and day (dual) ACTH secretory-burst waveform (left, top and bottom) and single-burst waveform (right, bottom) in nine healthy adults under control [Panel A] and metyrapone [Panel B] interventions. Statistical model comparison is made by the Akaiki Information Criterion (AIC) [right, top]. The latter defines a statistically preferable two-burst over one-burst model by a positive difference in the AIC pair (Methods), as observed here in 8 of 9 subjects in both feedback conditions. Asterisks on the x axis signify mean (of N = 9 individual)
quantiles (10%, 35%, 50% [median], 75%, 90%) of the time required to release the indicated percentage of a secretory burst; and, the centerpoint on the x axis marks the mean modal time delay to attain maximal secretion within a burst.

**Figure 7.** Stochastic features of the ACTH secretory burst-renewal process under control conditions (intact) and following metyrapone administration (low glucocorticoid feedback) according to the dual secretory-burst waveform model.  
*Panel A.* Probability distribution (Weibull density) of the set of interburst intervals (x-axis) in nine adults exposed to placebo (control, left) and metyrapone (right). The interrupted curve defines the distribution of pooled interpulse-interval lengths (min).  
*Panel B.* Scatterplot of maximum-likelihood estimates of paired ACTH secretory-burst frequency (x axis, lambda) and interpulse-interval regularity (y axis, gamma), based upon the likelihood function predicted by the Weibull probability distribution [Methods]. Central cohort estimates are shown as group median (plus sign) and pooled interpulse intervals (open circle).

**Figure 8.** Hypothesized mechanistic adaptations in pulsatile CRH and AVP release mediating hypocortisolemia-induced augmentation of both ACTH secretory-burst mass and frequency.  
*Top.* CRH, AVP and ACTH release in the cortisol feedback-intact state, wherein single arrows identify effectual CRH and/or AVP drive of ACTH pulses.  
*Bottom* (left to right). Low-cortisol feedback augmentation of CRH and/or AVP secretory-burst amplitude (or effectual [cortisol-disinhibited] signal strength), frequency or both, thereby eliciting larger and more frequent ACTH peaks (double arrows).
Table 1

Comparison of Day-Night ACTH Secretory-Burst Evolution in the Intact and Low Cortisol-Feedback State: dual waveform model

<table>
<thead>
<tr>
<th></th>
<th>Intact Feedback (control)</th>
<th>Low Feedback (metyrapone)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day</td>
<td>Night</td>
</tr>
<tr>
<td>Day/Night Interval Lengths (hr)</td>
<td>12.2 ± 1.1 [12.3]</td>
<td>11.8 ± 1.1 [11.7]</td>
</tr>
<tr>
<td>Regularity of Intervals²</td>
<td>2.68 ± 0.19 [2.58]</td>
<td>2.58 ± 0.39 [2.64]</td>
</tr>
<tr>
<td>Total Secretion (µg/L/24 hr)</td>
<td>0.93 ± 0.14 [1.0]</td>
<td>0.96 ± 0.09 [0.93]</td>
</tr>
<tr>
<td>Pulsatile Secretion (µg/L/24 hr)</td>
<td>0.65 ± 0.13 [0.63]</td>
<td>0.74 ± 0.08 [0.72]</td>
</tr>
<tr>
<td>Basal Secretion (µg/L/24 hr)</td>
<td>0.28 ± 0.09 [0.19]</td>
<td>0.23 ± 0.05 [0.24]</td>
</tr>
<tr>
<td>Mass per Burst (µg/L)</td>
<td>0.05 ± 0.01 [0.04]</td>
<td>0.08 ± 0.01 ** [0.08]</td>
</tr>
</tbody>
</table>

¹ lambda and ² gamma of Weibull distribution

Higher gamma (exceeding unity of the Poisson process) signifies greater regularity (CV less than 100%) of the set of interburst waiting times [Methods].

Data are the mean ± SEM [median] (N = 9).

* P = 0.02; ** P < 0.001; *** P < 0.005 by log-likelihood ratio testing of day vs night within a given feedback milieu.
Table 2

Time Unfolding of ACTH Secretory Bursts in the Intact and Low Glucocorticoid-Feedback State

<table>
<thead>
<tr>
<th>Analytical Waveform Feature</th>
<th>Day-Night Delta*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Time to secrete a given percentage of ACTH within a burst (min)</td>
<td></td>
</tr>
<tr>
<td>15%</td>
<td>-5.2± 8.4 (1)</td>
</tr>
<tr>
<td>35%</td>
<td>-20 ± 23 (8)</td>
</tr>
<tr>
<td>Median</td>
<td>-17 ± 26 (1)</td>
</tr>
<tr>
<td>75%</td>
<td>-13 ± 27 (-11)</td>
</tr>
<tr>
<td>90%</td>
<td>-18 ± 24 (0)</td>
</tr>
</tbody>
</table>

* within-subject difference (min)  ** P < 0.05 by paired t test

Data are the mean ± SEM (median) [N = 9].
References


8. Dayanithi, G and Antoni, FA. Rapid as well as delayed inhibitory effects of glucocorticoid hormones on pituitary adrenocorticotropic hormone release are mediated by type II glucocorticoid receptors and require newly synthesized messenger ribonucleic acid as well as protein. *Endocrinology* 125: 308-313, 1989.


29. Plotsky, PM and Sawchenko, PE. Hypophysial-portal plasma levels, median eminence content, and immunohistochemical staining of corticotropin-


40. Watanabe, T and Orth, DN. Detailed kinetic analysis of adrenocorticotropic secretion by dispersed anterior pituitary cells in a microperifusion system:
effects of ovine corticotropin-releasing factor and arginine vasopressin.


Reconstruction of Dual Secretory-Burst Waveform, Pulsatile and Basal Release and Biexponential Kinetics

- estimate pulse-onset times
- reconstruct single and dual waveform models*
- estimate burst mass, basal secretion, elimination rates and random effects simultaneously

* determine preferred model statistically via Akaike information coefficient
ACTH Secretory-Waveform Time Boundaries

Clock Time (h)

Placebo

Metyrapone

SUBJECT NUMBER

0638 h ± 23 min

1708 h ± 33 min

0659 h ± 25 min
DUAL ACTH SECRETORY-BURST MODEL: CONTROL

**Concentration**

- **SUBJECT A**
- measured line
- predicted line
- changepoints

**Secretion**

- **SUBJECT B**
- measured line
- predicted line

--- measured ---

--- predicted ---

--- day ---

--- day ---

Clock Time

Clock Time

Clock Time

Clock Time

- measured
- predicted

- measured
- predicted

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DUAL ACTH SECRETORY-BURST MODEL: METYRAPONE

SUBJECT A

Concentration

Clock Time

Metryapone

Control

changepoints

SUBJECT B

Metryapone

Control

SUBJECT A

Secretion

Clock Time

Metryapone

Control

SUBJECT B

Metryapone

Control

clocktimeClock Time

clocktimeClock Time

clocktimeClock Time

clocktimeClock Time

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Metyrapone

**ACTH Single Burst**
- measured
- predicted

**ACTH Dual Waveform**
- change points
- day
- night

---

[ACTH Single Burst](veldhuis\shared\slides\matlab\ACTH\Fig 4b.fig)

[ACTH Dual Waveform](veldhuis\shared\slides\matlab\ACTH\Fig 4b.fig)
**ACTH SECRETION: CONTROL**

**METYRAPONE**

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ACTH PULSE EVOLUTION: CONTROL

METYRAPONE
Control

ACTH DUAL WAVEFORM

MODEL COMPARISON

AIC (model contrast)

Time (min)

Subject

ACTH SINGLE BURST

Norm Secr Rate

Time (min)
Metyrapone

ACTH DUAL WAVEFORM

Day

Night

MODEL COMPARISON

Subject

ACTH SINGLE BURST

Norm Secr Rate vs Time (min)

Norm Secr Rate vs Day/Night

AIC (model contrast)

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DISTRIBUTION OF ACTH INTERBURST INTERVALS

CONTROL

METYRAPONE

Night

Day

Probability vs. Interburst Interval (min) for CONTROL and METYRAPONE conditions during Night and Day periods.

--- pooled
ACTH SECRETORY BURST-RENEWAL PROPERTIES

CONTROL

Night

O pooled
+ median

METYRAPONE

Night

Day

ACTH Secretory-Burst Frequency ($\lambda$)

Interburst-Interval Regularity ($\gamma$)
PUTATIVE MECHANISMS DRIVING AUGMENTATION OF ACTH SECRETORY-BURST MASS AND FREQUENCY

Intact Glucocorticoid Feedback

Baseline ACTH Pulses

Low Glucocorticoid Feedback

Amplitude

Frequency

Concordance

New ACTH Bursts

CRH

AVP

Baseline

Concentration

Time

Concentration

Time

Time