Endogenous ACTH concentration-dependent drive of pulsatile cortisol secretion in the human

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Keenan, Daniel M., Ferdinand Roelfsema, and Johannes D. Veldhuis. Endogenous ACTH concentration-dependent drive of pulsatile cortisol secretion in the human. Am J Physiol Endocrinol Metab 287: E652–E661, 2004. First published June 8, 2004; 10.1152/ajpendo.00167.2004.—According to current regulatory concepts, pulsatile ACTH concentrations (CON) stimulate time-lagged cortisol secretion rates (SEC) via an implicit CON-SEC dose-response relationship. The present analyses reconstruct nonlinear properties of this in vivo agonist-response interface noninvasively in order to investigate pulse-by-pulse coupling consistency and to obviate the need to infuse isotopes or exogenous effectors, which may disrupt pathway interactions. This approach required an ensemble strategy of 1) measuring ACTH and cortisol CON in plasma sampled every 10 min for 24 h in 32 healthy adults, and 2) estimating simultaneously a) variable-waveform ACTH and cortisol SEC bursts superimposed upon fixed basal SEC; b) biexponential kinetics of ACTH and cortisol disappearance; c) nonequilibrium exchange of cortisol among free and cortisol-binding globulin (CBG)- and albumin-bound moieties; d) two SEC-burst shapes demarcated by a statistically defined day/night boundary; e) feedforward efficacy, potency, and sensitivity; and f) stochastic variability in feedforward measures over time. Thereby, we estimate 1) ACTH SEC (µg·l⁻¹·day⁻¹) of 0.27 ± 0.04 basal and 0.87 ± 0.07 pulsatile (means ± SE); 2) cortisol SEC (µmol·l⁻¹·day⁻¹) of 0.10 ± 0.01 basal and 3.5 ± 0.20 pulsatile; 3) free cortisol half-lives (min) of 1.8 ± 0.20 (diffusion/advection) and 4.1 ± 0.30 (elimination) and a half-life of total cortisol of 49 ± 2.4 and of ACTH of 20 ± 1.3; 4) ACTH potency (EC₅₀, ng/l) of 26 ± 2.4, efficacy (nmol·l⁻¹·min⁻¹) 10 ± 1.8, and sensitivity (slope units) 0.65 ± 0.09; 5) night/day augmentation of ACTH and cortisol SEC-burst mass by 2.1- and 1.7-fold (median); 6) abbreviation of the modal time to maximal ACTH and cortisol SEC rates by 4.4- and 4.3-fold, respectively, after a change point clock time of 0205 (median); 7) in vivo percentage distribution of cortisol as 6% free, 14% albumin bound, and 80% CBG bound with an absolute free cortisol CON (nmol/l) 11.5 ± 0.54, and 8) significant (mean CV) stochastic variability in feedforward efficacy (140%), potency (38%), and sensitivity (56%) within the succession of paired ACTH/cortisol pulses of any given subject. In conclusion, the present composite formulation illustrates a platform for dissecting mechanisms of in vivo regulation of effector-response properties noninvasively in the corticotropic axis of the uninfused individual.

adrenal; feedback; pituitary; stress; diurnal; signaling

Experimental studies of the core mechanisms that maintain homeostasis in a neuroendocrine axis typically entail isolating one or more components. However, from an integrative vantage, interrupting signal exchange in an interlinked system limits valid assessment of intrinsic interactions (15, 37). A recent model-free statistical measure of coordinate network control is the approximate entropy (orderliness) of signal patterns (30). A complementary analytical strategy is estimation of in vivo dose-response relationships coupling effector concentrations (CON) to time-delayed glandular secretion rates (SEC) in a relevant model form (17, 18, 23). The motivation of the latter approach is estimation of physiological regulation of primary signaling interfaces without disrupting communication among the hypothalamus, pituitary gland, and target organ. Difficulties inherent in making such noninvasive assessments include 1) an unknown relative admixture of basal, pulsatile, and diurnal SEC activity; 2) a biological time delay between effector input and glandular output; 3) nonlinearity of the implicit effector-response function; 4) rapid transfer of ligand among free and distinct protein-bound compartments in plasma; and 5) stochastic elements associated with serial neurohormone measurements, the pulse-renewal process, the mass of hormone released per burst, and, potentially, parameters of the dose-response interface function (18, 20).

The present study builds on a recently validated technical platform intended to reconstruct probabilistic properties of pulsatile agonist CON-dependent drive of target-gland SEC noninvasively. We extend this foundation for the first time to examine endogenous time-varying control of a major signaling interface in the corticotropic axis in the uninfused healthy human.

Methods

Human Subjects

Conventional cross-correlation analysis of ACTH and cortisol CON time series was reported earlier (33). The present data do not overlap with earlier outcomes or methods in any manner. Briefly, 32 volunteers participated in the study (17 men and 15 women). Each subject provided written informed consent approved by the Institutional Review Board of the University of Leiden. The age range was 26–71 yr. Participants maintained conventional work and sleeping patterns and reported no recent (≤10 days) transmeridian travel, weight change (>2 kg in 6 wk), shift work, intercurrent psychosocial stress, prescription medication use, substance abuse, neuropsychiatric illness, or acute or chronic systemic disease. Complete medical history, physical examination, and screening tests of hematological,
renal, hepatic, metabolic, and endocrine function were normal. No subject had been exposed to glucocorticoids within the preceding several months.

Volunteers were admitted to the Study Unit the evening before sampling. The next day, a catheter was placed in a forearm vein at 0800. Ambulation was permitted to the lavatory only. Vigorous exercise, daytime sleep, snacks, caffeinated beverages, and cigarette smoking were disallowed. Meals were provided at 0800, 1230, and 1730, and room lights were turned off between 2200 and 2400 depending on individual sleeping habits. Blood samples (2.0 ml) were withdrawn at 10-min intervals from 0900 for 24 h. Specimens were collected in prechilled siliconized tubes containing EDTA (ACTH) or heparin (cortisol), centrifuged at 4°C to separate plasma, and frozen at −20°C within 30 min of collection. Total blood loss was <360 ml. Volunteers were compensated for the time spent in the study.

**Laboratory Assays**

Plasma ACTH concentrations were quantitated in duplicate by high-sensitivity (3 ng/l) and high-specificity double-monoclonal immunoradiometric assay using reagents from Nichols Diagnostics Institute (San Clemente, CA). Median within- and between- assay coefficients of variation (CVs) were 5.3 and 6.5%, respectively (33). Cortisol was assayed by high-sensitivity (25 nmol/l) solid-phase RIA (Sorin Biomedica, Milan, Italy). Intra- and interassay CVs were 5.1 and 6.4%, respectively (33). No samples were undetectable in either assay.

**Overview of Analytical Formulation**

The analytical objective is to estimate properties of the unmanipulated in vivo stimulus-response relationship mediating intermittent ACTH CON drive of time-delayed cortisol SEC over 24 h in individual subjects without administering an agonist, antagonist, or labeled marker (17, 18, 23). Dose-response parameter estimation proceeds simultaneously with prediction of basal and pulsatile CON and SEC time series, biexponential disappearance rates, and kinetics of free and bound ligand exchange in plasma.

The core model equations provide a composite representation of stochastic pulse timing (two-parameter renewal process); admixed basal and pulsatile SEC; hormone and subject-specific biexponential elimination kinetics; a flexible (three-parameter) SEC-burst waveform, which may be distinct in the day and night; random-effects on successive hormone SEC-burst mass; and, experimental uncertainty due to sample withdrawal, processing and assay (18, 23). Key features implemented here are highlighted below.

**Secretion and elimination functions.** The time-varying ACTH hormone CON, X(t), can be described by the solution to a set of coupled differential equations incorporating basal (time-invariant) and pulsatile (burst-like) SEC and biexponential elimination. Total secretion rate is given as the sum of basal and pulsatile: \( Z(t) = \beta_0 + P(t) \), with the resulting CON as follows

\[
X(t) = (ae^{-at} + (1-a)e^{-at})X(0) + \int_0^t (ae^{-a(t-r)} + (1-a)e^{-a(t-r)}) dr
\]

\[
Z(t)dr = \beta_0 \left( \frac{\alpha_1}{\alpha_1} (1 - e^{-at}) + \frac{1-a}{\alpha_2} (1 - e^{-at}) \right) + \int_0^t (ae^{-a(t-r)} + (1-a)e^{-a(t-r)}) dr
\]

where \( a \) is the proportion of rapid to total elimination, \( \alpha_1 \) and \( \alpha_2 \) the respective rate constants of the rapid and slow elimination phases, \( X(0) \) the starting hormone concentration, \( \beta_0 \) the basal SEC rate, \( t \) time, and \( P(t)dr \) the instantaneous pulsatile SEC rate over the infinitesimal time interval \( (r, r + dr) \) (18, 21, 23, 25). The function defining pulsatile SEC is given by

\[
P(t) = \sum_{j=1}^{\infty} M_j \phi(t - T_j^r), \ r \geq 0
\]

with

\[
M' = \eta_0 + \eta_1 \times (T_j^r - T_j^{r-1}) + \lambda
\]

and

\[
\phi(s) = \eta_2 e^{-|s|}, s \geq 0
\]

where \( \phi(s) \) denotes the waveform function (burst shape), a three-parameter generalized Gamma probability distribution normalized to integrate to unity; \( M' \) the (deterministic plus random) mass of hormone released per unit distribution volume in the \( j \) th burst; \( \eta_0 \) the fixed basal hormone synthesis rate in the SEC gland; \( \eta_1 \) the rate of additional mass accumulation over the time interval \( T_j^r - T_j^{r-1} \); and \( \lambda \) random effects on the mass of the \( j \) th SEC burst. Asymmetric and symmetric (e.g., Gaussian) SEC events are well approximated by the three-parameter Gamma density [above \( \phi(s) \) function]. Such flexibility is important, inasmuch as in vitro perfusion analyses of the impact of discrete secretagogue pulses on ACTH release disclose rapid onset and prolonged ACTH SEC after brief agonist exposure (1, 8). Analogously, in vivo sampling of adrenal venous effluent in the sheep, rat, and dog and in vitro perfusion of adrenal cells reveal temporal asymmetry of cortisol SEC under controlled ACTH drive (5, 13, 39).

The present construction allows for (but does not require) two SEC-burst shapes (waveforms or \( \phi \) functions), each expressed in an exclusive time window of the 24-h sampling period. The regions containing the independent pulse shapes are demarcated by a statistically identified change point (time boundary) (19, 23). The statistical validity of adding three parameters of pulse shape and a single change point time is tested by the Akaike information criterion (AIC). The AIC penalizes the reduction in the (negative logarithm of) the overall likelihood function (see APPENDIX) in proportion to the number of additional parameters evaluated at any given series length (19). A positive AIC difference between the single and dual waveform models in any given individual favors the dual SEC-burst model.

The total SEC rate is the sum of (diurnally fixed) basal and (variable) pulsatile release, given by \( Z(t) = \beta_0 + P(t) \). Allowable diurnal variations in SEC-burst frequency (number of bursts/unit time) and/or mass (CON units) provide the model-associated basis for the nycthemeral rhythm in observed hormone CON. Posture, hydration, and other unknown diurnal factors influence plasma cortisol-binding globulin (CBG) and albumin concentrations and thereby the putative cortisol (and in lesser measure ACTH) distribution space. The present 24-h analyses assume nominal mean values of each to facilitate multivariate calculations.

The present CON profile is a discrete time sampling of the foregoing underlying continuous processes plus random independently and identically distributed observational error (20, 22; APPENDIX).

**Model of interpulse-waiting times.** To incorporate flexible interpulse-interval variability about the probabilistic mean, we utilize the two-parameter Weibull (rather than one-parameter Poisson) renewal process. The Weibull distribution confers valid centered, normalized statistical representation of random-event occurrence with provision for potential (rare) extreme values. Then, the conditional probability density for any pulse time \( T^k \) given \( T^{k-1} \) is determined by

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

where \( \lambda \) designates the probabilistic mean frequency (expected average no. of events/unit time), and \( \gamma \) the regularity of interpulse waiting times. In the Weibull density, \( \gamma > 1 \) denotes greater regularity (lesser variability, CV < 100%) than in the Poisson model (wherein \( \gamma = 1 \)).
and the CV is fixed at 100% by construction). The mean, variance, and CV of the Weibull distribution are

\[
\begin{align*}
\text{Mean} &= \frac{1}{\lambda} \times \Gamma\left(1 + \frac{1}{\gamma}\right) \\
\text{Variance} &= \frac{1}{\lambda^2} \times \left[\Gamma\left(1 + \frac{2}{\gamma}\right) - \left(\Gamma\left(1 + \frac{1}{\gamma}\right)\right)^2\right] \\
\text{CV} &= \frac{1}{\Gamma\left(1 + \frac{1}{\gamma}\right)} - 1
\end{align*}
\]

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 interpulse-interval lengths depends expressly on \(\gamma\) (and not \(\lambda\), frequency).

**Nonlinear agonist-response interface function.** A statistical basis was presented and justified recently for nonlinear estimation of a four-parameter logistic function relating time-varying agonist CON to delayed glandular SEC in the presence of stochastic inputs (random perturbations) (17, 19, 23, 24; see Fig. 1A). This practicable dose-response structure is summarized in the APPENDIX. Experimental validation was accomplished by frequent (5-min) and extended (4- to 12-h) direct sampling of hypothalamo-pituitary portal and jugular venous blood in the unrestrained, conscious, unmedicated sheep and horse and repetitive sampling (every 20 min for 17 h) of LH and testosterone CON in the human spematic vein (17, 18). Statistical verification was by formal mathematical proof of unbiased asymptotic properties of maximum likelihood-based estimates (MLE) of all parameters in the system of equations (17, 20, 25).

**Figure 1.** Schematic representation of analytical concepts of relating each pulse of ACTH concentration (CON) to time-delayed cortisol secretion rates (SEC) (A) and estimating nonequilibrium exchange of secreted cortisol (Cort) among plasma free and albumin- and cortisol-binding globulin (CBG)-bound compartments vs. irreversible elimination (B).

**Ligand kinetics in plasma.** Nonequilibrium exchange of plasma cortisol among free and CBG- and albumin-bound moieties was modeled using \(I\) the set of coupled differential equations formulated initially for the distributional kinetics of testosterone among free and sex hormone-binding globulin-, and albumin-bound moieties (17); 2) general population mean (sex-independent) association (M \(^{-2}\)) and equilibrium dissociation constants (M) for cortisol-albumin of 3 \(\times\) \(10^3\) and 260 \(\times\) \(10^{-6}\) and for cortisol-CBG of 76 \(\times\) \(10^9\) and 39 \(\times\) \(10^{-7}\), respectively, as determined in human serum at \(37^\circ\)C and physiological pH (10, 12, 31); 3) a local population mean binding capacity (M) for albumin of 0.65 \(\times\) \(10^{-3}\) in both sexes and for CBG of 0.9 \(\times\) \(10^{-6}\) and 0.8 \(\times\) \(10^{-6}\) in women and men, respectively; and 4) half-lives for the off-rates for CBG and albumin of 0.9 SEC and 0.22 SEC, respectively. The possible fates of secreted cortisol under this model are illustrated schematically in Fig. 1B. The numerical representation of ligand exchange is given in APPENDIX.

**Primary model parameters.** Primary analytical outcomes of the current study include 1) basal SEC of ACTH (mg \(\times\) \(10^{-1}\), 24 h \(^{-1}\)) and cortisol (nmol \(\times\) \(10^{-1}\), 24 h \(^{-1}\)); 2) reconstructed (reconvolved) hormone CON and deconvolved hormone SEC time series; 3) rapid and slow-phase half-lives (min) of disappearance of plasma ACTH and free (protein-unbound) cortisol CON and the monoexponential half-life of total cortisol CON; 4) the mass of ACTH (mg) and cortisol (nmol) secreted per burst; 5) SEC-burst frequency (no. of discrete pulses per time interval, \(\lambda\) of the Weibull renewal process); 6) stochastic variability of interburst intervals (\(\gamma\) of the Weibull renewal process); 7) pulsatile SEC rate (product of mean SEC-burst mass and corresponding frequency); 8) change point (clock time ± min) of the SEC-burst waveform shift; 9) modal time latency (min) to attain maximal SEC within the (unit area-normalized) SEC burst; and 10) specific dose-response properties defined by \(I\) efficacy (asymptotically projected maximal ACTH-stimulated cortisol SEC, nmol \(\times\) \(10^{-1}\), min \(^{-1}\)); 2) potency (ACTH CON driving half-maximal ACTH SEC, EC \(_{50}\)); and 3) sensitivity (maximal positive slope of the ACTH CON-cortisol SEC relationship) (18–20, 23, 24). From a technical perspective, the foregoing ensemble of parameters is evaluated simultaneously, conditioned statistically on a priori estimates of ACTH pulse-onset times, as described (20). In this simplified model, cortisol SEC bursts inherit pulse times from ACTH. Mass units are normalized per liter of distribution volume, given that reported cortisol distribution volumes vary absolutely from 4.8 to 44 l (nominal literature minimum \(7\) l/m\(^2\)) (31).

**Stochastic dose-response perturbation.** Stochastic variations about the deterministic mean dose-response function were permitted, but not required, to unfold on a pulse-by-pulse basis within any given paired 24-h ACTH CON-cortisol SEC time series (17). On statistical grounds, stochastic fluctuations were allowable in any one of efficacy, potency, or sensitivity (APPENDIX). The analytical intent is faithful representation of biological nonuniformity of the ACTH CON-cortisol SEC relationship in a given train of paired pulses in a particular subject over 24 h. Stochastic variability was defined quantitatively by the CV of within-subject estimates of dose-response efficacy, potency, or sensitivity on a pulse-by-pulse basis.

**Statistical Analyses**

Data are given as means ± SE [median]. Student’s *t*-statistic was used in exploratory comparison of SEC and kinetic parameters by sex. Linear regression analysis was applied to relate parameter estimates to age. Significance was construed for two-tailed \(P < 0.05\).

**RESULTS**

Mean (24-h) plasma ACTH CON were 19 ± 0.71 [17] ng/l and cortisol CON 245 ± 11 [227] nmol/l for the combined group \((n = 32)\). Gender did not influence either measure. Figure 2 illustrates a plasma ACTH CON time series based on
sampling every 10 min for 24 h in one volunteer. To aid in visualizing model performance, plots depict each of observed (directly measured) ACTH CON, statistically reconstructed time-varying ACTH CON (analytically reconvolved fit of each profile), a priori estimation of individual pulse-onset times for ACTH, estimated instantaneous ACTH SEC, probabilistic definition of the three-parameter waveform of ACTH and cortisol SEC bursts, and statistical representation of the single and dual SEC-burst models for ACTH with the analytically identified change point (time of the day/night shift in secretory-burst shape).

Figure 3 provides statistical estimates of the interburst interval (min) and the mass of ACTH secreted per burst (ng/l) and each of basal, pulsatile, and total ACTH SEC in the combined cohort. Segmentation between day and night is based on an analytically defined ACTH SEC-burst waveform change point (clock time) estimated to fall at 0150 (median 0205 h). The corresponding night/day change point time for ACTH was 1545 (median). Sex and age did not influence any of the foregoing measures significantly. Both the frequency and statistical regularity of interpulse-waiting times (of Weibull renewal process) were similar in the analytically defined night and day: (a) [median] lambda 22 and 20, and gamma 2.44 and 2.60, respectively. Daily median independently of night/day segmentation were 21 bursts/24 h.
and 2.46, respectively. A γ value exceeding unity denotes greater regularity (lower CV) than that of a Poisson process (CV fixed at 100%).

Figure 4 depicts the probabilistic waveform of ACTH and cortisol SEC bursts (time evolution of instantaneous hormone SEC rates within an underlying pulse) in three individuals. Cortisol SEC is presented in relation to each of the three stochastic models of dose-response estimation, which may transduce any given ACTH input signal (see METHODS). The analytically reconstructed SEC-burst waveform is a normalized probability function that integrates to unity. Thus the waveform is independent of the mass of hormone contained in the burst. This feature permits valid comparison of waveform evolution based upon the modal time to attain maximal SEC (see below).

Figure 5A summarizes analytical estimates of the mode (min) of the daytime (before change point) and nighttime (after change point) time latency to maximal ACTH and cortisol SEC within discrete bursts in men and women. ACTH and cortisol SEC bursts evolved slowly in the day (time range of mode, 108–116 min for both) and rapidly at night (range 22–30 min). Concomitantly, ACTH and cortisol SEC-burst mass values were respectively 2.1- and 1.7-fold (median) higher in the night than in the day. The algebraic mean within-subject day-night increment was significantly positive in the entire cohort (P < 0.001; Fig. 5B). In the potency model, the absolute day-night increment in cortisol SEC-burst mass was significantly (~2-fold) greater in men than in women. The efficacy and sensitivity models yielded similar trends.

The ACTH half-life (slow phase) averaged 20 ± 1.3 min. The rapid phase approximated the least determinable half-life for this (10-min) sampling frequency (i.e., 6.93 min) (19). In relation to cortisol, stochastic model type did not influence predicted half-lives (min). Mean values across the three models were for free cortisol (1.8 ± 0.20 rapid and 4.1 ± 0.30 slow phases) and for total cortisol (50 ± 2.1). These values reflect statistical estimation of cortisol exchange among free and albumin- and CBG-bound compartments based on population of mean rates of dissociation and association (see METHODS and Fig. 6). The percentages of estimated free and albumin- and CBG-bound cortisol in plasma were 6, 14, and 80, respectively.

Figure 7 depicts the results of analytical reconstruction of four-parameter dose-response functions linking pulsatile ACTH CON to time-delayed cortisol SEC in one subject. Data are given for each of the three stochastic constructs. Individual curves for a given model form represent inferred stochastic

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**Fig. 4.** Three-parameter waveform (generalized Gamma density) encapsulating the time evolution of the normalized rate of instantaneous ACTH (top) and cortisol (bottom) SEC within discrete bursts. The cortisol waveform is the application of the cortisol dose-response function to the ACTH waveform. Separate panels in a given row represent data from an individual adult. The 3 cortisol waveform curves in each of the bottom panels give outcomes for the distinct stochastic versions of dose-response estimation. For ease of visualization, only nighttime burst shapes are illustrated.

**Fig. 5.** Mass of cortisol secreted per burst (nmol/l) in the statistical day and night under the 3 stochastic dose-response models. Data are means ± SE; n = 32 subjects.
pulse-by-pulse variability in coupling properties evolving over 24 h in the unstressed individual. A single 24-h profile of (model-independent) ACTH CON signals is given, because stochastic elements influence ACTH burst mass and timing only. Three 24-h cortisol CON time series are reconstructed, because stochastic variability is allowed for each of the three primary parameters of the dose-response transduction process (ACTH signal driving cortisol SEC and, hence, convolved cortisol CON). Realizations of ACTH and cortisol CON profiles and 24-h cortisol SEC are rendered above each stochastic dose-response model.

Figure 8 gives reconstructed four-parameter ACTH cortisol SEC dose-response functions associated with each stochastic model in individual subjects (n = 32). Table 1 compares model-specific predictions for each of the primary dose-response measures: efficacy (asymptotically maximal cortisol SEC rate, nmol·1−1·min−1), potency (EC50, ng/l), responsivity SEC50 (one-half maximal cortisol SEC rate), and sensitivity (slope of calculated dose-response function).

Analytical parameter estimates for any given paired stimuluss-secretion pulse in one subject are well determined (CV < 8.5%). As verified experimentally recently for LH-testosterone pulse coupling, successive effector-response relationships in any subject vary stochastically over time (17, 24). The present analyses unveil the same properties for ACTH/cortisol pulse pairs. In particular, stochastic variability over 24 h averaged 70% for efficacy, potency, and sensitivity. Under the same models, (24-h mean) free cortisol CON estimates were 11.4 ± 0.52, 11.5 ± 0.50 and 11.3 ± 0.51 nmol/l [P = not significant (NS) by model form].

**DISCUSSION**

The present biomathematical analyses reconstruct deterministic and stochastic properties of the nonlinear dose-response function putatively coupling endogenously pulsatile ACTH CON to time-varying cortisol SEC in 32 healthy adults by use of a noninvasive strategy. The intent of developing a noninvasive analytical means to assess in vivo dose responsiveness is to 1) allow assessment of pulse-by-pulse ACTH/cortisol coupling variability and 2) complement classical methods requiring infusion of isotopes or other marked chemicals, some of which may disrupt the interactive pathway(s) under study (17, 24). On the basis of the accompanying statistical formalism, the current formulation predicts 1) twofold augmentation of the amount of ACTH and cortisol secreted per burst in the statistically demarcated night compared with day; 2) fourfold abbreviation of the time evolution of ACTH and cortisol SEC bursts in the analytical night compared with day; 3) ACTH CON-dependent free of cortisol SEC via an inferentially cooperative and asymptotic feedforward dose-response relationship that operates within the midphysiological agonist CON range (ACTH EC50 26 ± 2.4 vs. mean ACTH CON 19 ± 0.71 ng/l); 4) in vivo percentage distribution of total cortisol as 6% free and 14% albumin- and 80% CBG-bound with an absolute (24-h mean) free cortisol CON estimate of 11.5 ± 0.54 nmol/l; and 5) significant (mean CV) apparently random variability in stimulus-response efficacy (145%), potency (38%), or sensitivity (56%) within any given set of paired 24-h ACTH and cortisol pulses compared with <8.5% for a single ACTH/cortisol pulse pair. Thus successive dose-response curves defined over 24 h are not static.

The present study in healthy adults illustrates a new strategy for analytical reconstruction of time-evolving ACTH/cortisol dose-response properties. A hallmark of this approach is noninvasive application to uninfused, unblocked, and unstimulated individuals, thereby permitting appraisal of physiological regulation in vivo. The basic experimental requirements are 1) frequent serial measurements of paired input (effector) and output (response) CON and 2) simultaneous statistical estimation of all parameters of basal and pulsatile SEC, biexponential elimination kinetics, free and bound ligand exchange, and dose-response efficacy, potency, and sensitivity. The statistical solution is an asymptotically maximum-likelihood estimate predicated on a priori-estimated pulse times (see METHODS). According to this analytical platform, in vivo ACTH CON-dependent stimulation of cortisol SEC in men and women is characterized by 1) principally pulsatile ACTH (>75%) and cortisol (>95%) SEC; 2) a (median) half-life of ACTH of 19 min and of total cortisol of 49 min and half-lives of free cortisol of 1.8 min (rapid phase) and 3.5 min (slow phase); and 3) estimated SEC rates of ACTH of 1.1 ± 0.6 μg·l−1·day−1 (nominally 3.9 μg·person−1·day−1 for a distribution volume.
of 3.5 liters) and of cortisol of $3.6 \pm 0.2 \, \mu \text{mol} \cdot \text{l}^{-1} \cdot \text{day}^{-1}$ ($\sim 12.8 \, \text{mg} \cdot \text{person}^{-1} \cdot \text{day}^{-1}$ for a distribution volume of 10 liters). The foregoing estimates are consistent with values, where available, determined directly by isotope infusion in vivo or estimated by equilibrium dialysis at 37°C, pH 7.4, in vitro (6, 10, 12, 23, 26, 27, 31–33).

As inferred recently for TSH (19), the predicted waveform of ACTH and cortisol SEC bursts differs markedly in the statistically demarcated day and night. In particular, SEC proceeds more rapidly at the outset of a release episode in the objective night than in the day for ACTH and cortisol. An ACTH waveform transition was also inferred earlier in a smaller number of volunteers evaluated in the cortisol feedback-depleted vs. feedback-intact state (23). For ACTH, the analytically defined median day/night secretory-burst shape change point was 0205. The night/day waveform change point occurred 13 h 40 min later (at 1545 clock time). The former estimate corresponds to shortly before the absolute 24-h nadir in cortisol secretion (nominally 0430) identified independently earlier (40). The current analytical definition of a day/night boundary must be distinguished from that of the time of the maximum (acrophase) of a 24-h sinusoidal function (cosinor analysis). Objective justification of day/night segmentation requires statistical penalty of the enhanced precision of fit (negative log-likelihood function) of ACTH CON profiles due to adding four additional parameters, one designating the change point time and three others encapsulating the second SEC-burst shape (19, 23). According to the AIC (41), 30 of 32 ACTH time series were represented more favorably by a dual than a single waveform ($P < 10^{-5}$ by sign test). Further analysis suggested a possible sex difference in the diurnally modulated cortisol SEC response; i.e., a 1.9-fold greater night/day increment in the mass of cortisol secreted per burst in men than in women. The analytical day and night did not differ in ACTH SEC-burst frequency normalized per hour (lambda of Weibull process) or interpulse-
interval regularity (gamma of the Weibull distribution). Plausible mechanisms driving high-mass ACTH and cortisol SEC bursts at night could respectively include 1) lesser cortisol negative feedback and/or greater secretagogue feedforward on the hypothalamo-pituitary unit and 2) accentuated responsiveness of adrenal cortisol SEC to ACTH stimulation (2, 11).

Laboratory studies more than four decades ago identified greater adrenal weight, higher ACTH and corticosterone CON, and elevated basal and stress-stimulated glucocorticoid SEC in the adult female compared with the male rat (3, 7). In this regard, some clinical studies have reported a lower ratio of the adult female compared with the male rat (3, 7). In this context, some clinical studies have reported a lower ratio of the adult female compared with the male rat (3, 7).

In contradistinction, direct measurements of urinary free cortisol synthesis and elevate total cortisol CON (14, 16, 34, 35). In this context, some clinical studies have reported a lower ratio of the adult female compared with the male rat (3, 7).

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The accompanying projections of ACTH potency (11–34 ng/l, dependent on stochastic model) are concordant with inspection and reploting of the relationships between l) peak injected ACTH CON and cortisol CON and 2) corticotropin-releasing hormone-stimulated ACTH CON and incremental cortisol CON reported elsewhere (28, 29). Inferred pulse-by-pulse stochastic variability about the deterministic mean of any one of the three principal dose-response parameters (efficacy, potency, and sensitivity) was 3- to 10-fold intra-assay variability. Variability of the dose-response relationship arises within the set of ACTH/cortisol pulse pairs observed over 24 h in any one subject. In contrast, the CV of parameter estimates is <8.5% when determined for a single ACTH/cortisol pulse pair. The precise basis for apparent fluctuations of in vivo feedforward coupling within a 24-h pulse train is not known. Plausible explanations include, first, recurrent epochs of partial sensitization and desensitization of adrenal responsiveness to randomly timed and nonuniform ACTH pulses, as postulated recently for LH-testosterone coupling (24). Moreover, even at a fixed frequency and dose, repeated ACTH stimuli successively augment and suppress corticosterone SEC by perifused rat adrenocortical cells monitored by in vitro perfusion (36). Second, oscillations in adrenal blood flow that are partially dependent on ACTH stimulation may influence cortisol SEC rates in vivo (5, 38). And third, multiple systemically derived and intra-adrenally synthesized factors either facilitate or repress ACTH-stimulated steroidogenesis (11). Whether these or other mechanisms contribute to apparent stochastic fluctuations in specific dose-response parameters about the deterministic mean parameter value over 24 h in any one subject is not known.

In summary, the accompanying analyses illustrate a statistical strategy for simultaneous estimation of 1) the secretion and elimination kinetics of ACTH and free and total cortisol noninvasively and 2) deterministic and stochastic properties of pulsatile ACTH CON-dependent stimulation of cortisol SEC in the normal human.

**Perspectives**

Further combined analytical and experimental investigations of mechanisms underlying integrative control of the corticotropic axis might reasonably include noninvasive estimation of in vivo dose-response properties subserving 1) pulsatile hypothalamic secretagogue drive of pituitary ACTH SEC and 2) free, bioavailable, and/or total cortisol CON-dependent feedback on the hypothalamo-pituitary unit. Because the stress-adaptive corticotropic axis is a prototypical interlinked network, the accompanying biomathematical formalism should also have utility in appraising the regulation of nonlinear coupling of paired signals in other neuroendocrine systems.

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**Table 1. Analytical estimates of ACTH CON-cortisol SEC dose-response parameters under 3 stochastic models**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Efficacy</th>
<th>Potency</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC50, ng/l</td>
<td>11±2.1 [9.9]</td>
<td>2.6±0.66 [1.3]</td>
<td>0.02±0.14 [0.40]</td>
</tr>
<tr>
<td>Cort Sec50, nmol/l/min</td>
<td>5.3±1.3 [2.6]</td>
<td>13.0±0.67 [0.13]</td>
<td>0.17±0.02 [0.14]</td>
</tr>
<tr>
<td>Efficacy, nmol/l/min</td>
<td>1.0±2.6 [0.40]</td>
<td>0.02±0.14 [0.40]</td>
<td>0.65±0.09 [0.45]</td>
</tr>
</tbody>
</table>

Data are means ± SE [median] in 32 adults. Cort Sec50, one-half maximal cortisol secretion rate.
APPENDIX

A. Formulation of Feedforward Interface Properties

Agonism, $F_A(t)$, is enforced via a delayed time-averaging of ACTH CON, $X_A$, wherein time delays are $1, 2$

$$ F_A(t) = \frac{1}{1} \int_{t-1}^{t-2} X_A(\tau) d\tau $$

[Note: In METHODS, ACTH SEC was modeled (e.g., Eq. 1), and there was no need to distinguish between ACTH and cortisol. Here, the subscript, A, will be used to identify ACTH, e.g., $X_A$. Random effects are also denoted by the symbol $A$, but the distinction is stated in each context.] The ACTH CON signal drives free cortisol SEC, $Z_C(t)$, via a four-parameter logistic dose-response function (below). A theoretical justification for this general structure has been suggested earlier (9). The present analyses allow for possible burst-to-burst stochastic variability, $A_C^j$, in feedforward efficacy, sensitivity or potency (defined in METHODS). The nonlinear dose-response interface functions that link the ACTH CON signal, $F_A(t)$, to the cortisol SEC rate, $Z_C(t)$, for each of the three separate stochastic models are given by

$$ Z_C(t) = \begin{cases} \frac{\eta_{1C} + A_C}{1 + \exp[-(\eta_{1C} + \eta_{1C} \times F_A(t))]}, & t < T_A^* \ , j = 1, \ldots, m \\ \text{[allowable variation (A_C's) in upper asymptote: efficacy]} \end{cases} $$

C. Realization of Con Series

Hormone CON series with superimposed experimental uncertainty, $e_i(t)$, are defined by

$$ Y_{k,i} = X_i(t) + e_i(t), \ i = 1, \ldots, n, k = \text{ACTH} (A) \text{ and cortisol} (C) $$

where $X_i(t)$ is the output of the combined SEC and elimination function. The expression for $X_C$ is given above and that for $X_A$ from (Eq. 1 in METHODS)

$$ X_A(t) = [a_0 e^{-\alpha C} + (1 - a_0) e^{-\alpha C} Z_A(0)] + \int_0^T [a_0 e^{-\alpha C} + (1 - a_0) e^{-\alpha C}] Z_A(\tau)d\tau $$

D. Variance Estimation

For analytical reconstruction of ACTH SEC and variable efficacy of the dose-response function, random elements ($A_A$, $A_C$) enter into SEC rates linearly; thus the maximum likelihood estimate, $\theta$, of the parameter set is obtained by maximizing the corresponding Gaussian likelihood function. For statistical estimation of variable dose-response sensitivity and potency, random elements contribute nonlinearly to cortisol SEC. Here, the solution is achieved iteratively in two stages: 1) random effects are held fixed, and dose-response parameters are maximized (as above); and 2) parameters are held fixed, and conditional expectations of the Gaussian random effects are obtained via an expectation-maximization algorithm. SEC rates are then conditional expectations evaluated at the MLE, $\theta$

$$ Z_A(i = 1, \ldots, n) = E_{\theta} \ [Z_A(t), i = 1, \ldots, n | Y_{k,i} = 1, \ldots, n] $$

early to cortisol SEC. Here, the solution is achieved iteratively in two stages: 1) random effects are held fixed, and dose-response parameters are maximized (as above); and 2) parameters are held fixed, and conditional expectations of the Gaussian random effects are obtained via an expectation-maximization algorithm. SEC rates are then conditional expectations evaluated at the MLE, $\theta$

$$ Z_A(i = 1, \ldots, n) = E_{\theta} \ [Z_A(t), i = 1, \ldots, n | Y_{k,i} = 1, \ldots, n] $$

$$ k = \text{ACTH and cortisol} $$

A convolution of SEC rates with biexponential kinetics, a linear procedure, yields fitted (predicted) CON: $\hat{Y}_{k,i} = 1, \ldots, n, k = \text{ACTH and cortisol}$

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