Dose-Response Downregulation within the Span of Single Interpulse Intervals

Daniel M. Keenan¹
Ferdinand Roelfsema²
Johannes D. Veldhuis³*

¹Department of Statistics
University of Virginia
Charlottesville, VA  22908

²Department of Endocrine and Metabolic Diseases
Leiden University Medical Center
2333 ZA Leiden
The Netherlands

³Endocrine Research Unit
Mayo School of Graduate Medical Education
Center for Translational Science Activities
Mayo Clinic
Rochester, MN 55905

*Corresponding author
Short head: Dose-response autoregulation

Key words: feedforward, feedback, model, stress, human, endocrine, systems biology

Word counts: Abstract: 292; Text: 2543; Appendix 1116

Number of references: 40; Tables: 1; Figures: 6
Abstract

Pituitary adrenocorticotropin (ACTH) drives adrenal glucocorticoid (cortisol) pulses via a time-delayed asymptotic dose-response process. To test the postulate that ACTH stimulates cortisol secretion dynamically (unequally during the initiation and termination of a cortisol secretory burst), a mathematical formalism was developed in which dose-response hysteretic shifts were allowed, but not required, within the time evolution of ACTH-cortisol pulse pairs. A dual-waveform deconvolution model was used to quantify cortisol secretion rates and reconstruct ACTH concentration profiles in 28 healthy adults previously sampled every 10 min for 24 hr in the unstressed state (8,120 measurements). ACTH concentration-cortisol secretion dose-response functions were then estimated in each subject (i) without hysteresis (base model), and with allowances for possible hystereses in (ii) ACTH potency (iii) adrenal sensitivity and (iv) ACTH efficacy. Model residual error was 40% lower in the potency and sensitivity models and 20% lower in the efficacy model than in the base model (P < 0.001). Mean time shifts for inferable hysteretic inflection were model-independent, viz., grand mean (95% confidence interval) 22 (12-39) min after the onset of a cortisol secretory burst. Half-maximally effective ACTH concentrations (EC_{50}) differed before and after hysteretic inflection within individual pulses: (a) 9.4 and 54 ng/L in the potency model (P < 0.001); and (b) 8.9 and 123 ng/L in the sensitivity model (P < 0.001) compared with 16 ng/L for the no-hysteresis model (P < 0.001). In the efficacy-shift model, estimated maximal ACTH drive varied by 17-fold within cortisol secretory bursts (from 22 to 1.3 nmol/L/min cortisol secretion P < 0.001). The collective results introduce the basis for modeling the dynamics of rapid, reversible physiological downregulation within the span of single
interpulse intervals \textit{in vivo}. This construct should have utility in parsing mechanisms of physiological regulation in other integrative systems.

\textbf{Word Count:} 292
**Introduction**

Physiological systems maintain homeostasis by reciprocal feedback (inhibitory) and feedforward (stimulatory) interactions among critical regulatory nodes (4). Typical examples include the cardiovascular-baroreceptor, respiratory-chemoreceptor, and diverse neuroendocrine systems. Intermittent time-delimited neuroendocrine signal exchange mediates adaptations in reproductive, metabolic, growth-related and stress-activated axes (39). Local and blood-borne signals in turn act via implicit nonlinear dose-response functions, which transduce repeated incremental adjustments toward physiological optima (18; 38). Homeostatic control in biological networks is viewed as proceeding via such time-delayed, nonlinear, asymptotic adjustments to varying internal and external signals (14; 15).

Recent investigations in neuroendocrine ensembles suggest that finite random (stochastic) variability exists in biological parameters, beyond *de facto* imprecision in experimental measurements (13; 14; 16). Biological variability may arise from nonuniformities in gene expression, glandular secretion, circulatory distribution (advection and diffusion), and fractional elimination (metabolism and biotransformation) of hormones, ligands, metabolites and effectors (3; 27; 34; 38). In addition, both the amplitude and the timing of successive secretory bursts is episodic and uncorrelated, typical of a random renewal process (14; 15).

A plausible contributor to inferably stochastic pulse-to-pulse variations would be short-term random variability in effector potency, efficacy or target-gland sensitivity. Indeed, in principle, seemingly random variability in serial secretory-burst size might reflect cycles of deterministic (biologically specified) response desensitization and/or
resensitization occurring within or between consecutive secretory bursts. If so, an effector and target gland-selective time interval (or range of intervals) should exist within which hypothesized down- or upregulation evolves. Physiological cycles of reversible response suppression or enhancement could be consistent with a wide repertoire of other pharmacological observations in vitro and in vivo (1; 2; 10; 26; 32).

The present analyses introduce a quantitative platform for interrogating paired pulse trains for possible (mathematically estimable) cycles of dose-dependent downregulation and/or upregulation during the time evolution of individual pulses. The outcomes suggest a novel mode of dynamic signal regulation and integration.

Methods

Human subjects

Conventional cross-correlation, approximate entropy, and dose-response evaluation of these ACTH and cortisol time series were reported earlier (16; 30). The present analyses of these archival data do not overlap with earlier outcomes or methods. Briefly, 28 volunteers participated in the study (13 men and 15 women), after supplying informed consent approved by the Leiden University Institutional Review Board. The age range was 30-77 yr, and body-mass-index range 18-30 kg/m². Participants had conventional work and sleep patterns. None had a recent history of shift work, international travel, weight change, depression, psychosis, untoward stress, alcohol or drug abuse, neuropsychiatric medication exposure, acute illness, systemic disease, or glucocorticoid use. Screening tests of general health were normal (30).

Volunteers were admitted to the Leiden Study Unit for overnight adaptation before undergoing frequent blood sampling beginning at 0900 hr the next day (30). Blood was
collected every 10 min for 24 hr into ice-cold siliconized tubes containing EDTA (ACTH) or heparin (cortisol), centrifuged in the cold, and frozen within 30 min of collection, as described (30).

**Laboratory assays**

Plasma ACTH concentrations were quantified in duplicate as described originally using an immunoradiometric assay [Nichols Diagnostics Institute (San Clemente, CA)] (30). Plasma cortisol was measured by RIA [Sorin Biomedica, Milan, Italy] (30).

**Overview of analytical formulation**

The analytical objective was to estimate possible cycles of *in vivo* desensitization (or resensitization) associated with the stimulus-response (feedforward) relationship mediating pulsatile ACTH concentration-dependent drive of time-delayed cortisol secretion. This has never been accomplished using these archival (or any other) data sets. The core model equations, which were developed earlier (14; 15), together embody stochastic pulse timing (two-parameter Weibull renewal process); admixed basal and pulsatile secretion; hormone and subject-specific biexponential elimination kinetics; a flexible (three-parameter generalized Gamma distribution) secretory-burst waveform or shape; random-effects on successive hormone secretory-burst mass; and, experimental uncertainty due to sample withdrawal, processing and assay (15; 16). To optimize deconvolution analysis, the model allowed for (but did not require) two secretory-burst shapes (waveforms or psi functions), each expressed in an exclusive continuous time window in the 24-hr sampling period, as described earlier for another data set (17). Key new features implemented in the present framework are presented in the **Appendix**, and highlighted below.
Nonlinear effector-response function

The core model is a four-parameter logistic function relating time-varying agonist concentrations to delayed glandular secretion rates in the presence of finite stochastic inputs (random perturbations) (3; 14). Specific dose-response properties are defined by the following parameters: (i) efficacy (asymptotically projected maximal ACTH-stimulated cortisol secretion rate, nmol/L/min); (ii) potency (an exponential measure related inversely to the ACTH concentration driving one half-maximal ACTH secretion, \( EC_{50} \)); (iii) sensitivity (maximal positive slope of the ACTH-cortisol dose-response relationship); and (iv) basal (nonpulsatile) cortisol secretion (15; 16). The innovation is to extend this structure to allow for three possible types of dose-response hystereses models, comprising possible intrapulse hysteresis-like shifts in potency, sensitivity or efficacy. Hysteresis was defined here as an effector-response dynamic, in which initial and delayed stimulation parameters over time within any given pulse differ with respect to any one of potency, sensitivity or efficacy: Figure 1. Hysteresis is permitted (the dose-response curve is allowed to shift) within a finite positive time interval after the deconvolution-based onset of each cortisol secretory burst. The time-delay interval is estimated simultaneously with the dose-response parameters. ACTH stimulation before this time operates via one family of dose-response functions, and after this time via another (hysteretic-recovery) family of dose-response functions. Each model, including the no-hysteresis base model allows for possible random effects on cortisol secretory-burst mass (14), here designed \( \delta_A \). Residual model error, \( \delta_e \), is estimated concurrently from comparison of model-predicted and deconvolution-estimated (cortisol) sample secretory rates.
Statistical Analyses

One-way ANCOVA with 3 categorical factors (the 3 hystereses models) was the primary statistical model, wherein the no-hysteresis (base) model output served as the covariate. Data were first transformed by taking the natural logarithm. Thus, parameters of the model are given in tables as the geometric mean (95% confidence interval), and in figures as box-and-whisker plots. Other data are reported as the arithmetic mean ± SEM or median (range), as noted. Post hoc Tukey’s honestly significantly different test was used for multiple comparisons at experiment-wise protected P ≤ 0.05. ANCOVA results were confirmed by the Kruskal-Wallis nonparametric test. The Student’s two-tailed, unpaired, common-variance t-statistic was used to compare dose-response parameters by gender at protected P ≤ 0.01. Linear regression analysis was applied to explore the relationship of parameter estimates to age and BMI.

Results

Figure 1 schematizes the 3 dynamic models of effector dose-secretory responses proposed (Methods) and examined here. The 3 separate hystereses constructs allow, but do not require, the operation within each interpulse interval of two possible potencies, two possible sensitivities or two possible efficacies. The hysteretic switch time (reflection point) was estimated simultaneously with all parameters of the dose-response functions (see Appendix).

As a first step, ACTH and cortisol concentration-time series were deconvolved via the dual-waveform secretory-burst model (16; 17). The unit-area normalized shape of secretory bursts (plot of rate of secretion over time) was permitted to differ in the day
and night, thus constituting a dual-waveform model of secretion. Two changepoint times were estimated to demarcate onset of the day and onset of the nighttime waveforms within each 24-hr pulse train (as discussed fully in (17). Figure 2 illustrates outcomes of this first step in one subject. The effector signal (dose-response input) was defined as the time-shifted reconvolution (fitted) curve for ACTH concentrations. The dose-response output was taken as (deconvolved) cortisol secretion rates. The resultant 145 concentration-secretion pairs in each subject were related via a 4-parameter logistic dose-response function without hysteresis (base model); a 5-parameter hysteretic potency model (separate ACTH potencies for initial and delayed segments of cortisol-secretory bursts); and the latter structure, but embodying either two sensitivities or two efficacies: Figure 3. ANCOVA of model residual errors (using the no-hysteresis model residual error as the covariate) revealed a descending order of goodness-of-fit as follows in the set of 28 subjects: potency hysteresis = sensitivity hysteresis > efficacy hysteresis > no hysteresis (overall \( P < 0.001 \), covariate effect \( P < 0.001 \)). Nonparametric Kruskal-Wallis testing corroborated the model distinctions \( (P < 0.001) \). In particular, model residual error was 40% lower in both the potency and sensitivity models, and 20% lower in the efficacy model, than that in the nonhysteresis (base) model. For the 3 hystereses models, initial and delayed sensitivity differed by 13.7-fold (sensitivity model), potency by 5.6-fold (potency model), and efficacy by 17-fold (efficacy model) \( (each \ P < 0.001) \). In each case estimates of hysteretic dose-response parameters flanked those of the no-hysteresis model. Table 1 summarizes these comparisons (rows 1 and 2). In contradistinction, mean time delays for hysteresis were comparable among the 3 dose-response models at 22 (12-39) min (geometric
mean [95% confidence interval], P = 0.962 by ANCOVA, P = 0.878 by KW test) [row 3, Table 1].

Parameter estimates in the 3 hystereses models were compared by ANCOVA. Mean basal (nonpulsatile) cortisol secretion was about 6-fold lower in the efficacy hysteresis construct than in the other 3 models (row 4, Table 1, P < 0.001). The exponential parameters for potency and sensitivity, and the numerator term for efficacy, differed by hysteresis model (each P ≤ 0.001): Figure 4. In particular, mean absolute (unsigned) potency was about 3-fold higher in the no-hysteresis and sensitivity models than that in either the potency or efficacy models (P < 0.001) [left panel, Figure 4]. ACTH sensitivity was highest in the sensitivity model (P < 0.001), intermediate in the no-hysteresis and efficacy models, and lowest in the potency model (P < 0.001) [middle panel, Figure 4]. ACTH efficacy was higher in the potency and efficacy models than in the no-hysteresis and sensitivity constructs (P = 0.001) [right panel, Figure 4].

Inasmuch as the EC50 for ACTH drive is defined numerically as -potency/sensitivity, EC50 provides an ensemble measure of submaximal stimulatory effects. Figure 5 depicts EC50 values (ng/L ACTH concentrations) associated with each of the 3 hystereses models compared with the no-hysteresis base model (interrupted horizontal lines). The base model with two allowable secretory-burst shapes yielded a geometric mean EC50 of 16 ng/L (and an arithmetic mean ± SEM of 23 ± 3.3 ng/L), compared with 24 ± 2.1 ng/L (arithmetic mean) in an earlier estimate using a nonhysteresis dose-response model (16). In the potency hysteresis construct, initial and delayed EC50’s were 9.4 and 54 ng/L (P < 0.001 by Tukey’s test). The initial EC50 (8.9 ng/L) in the sensitivity model was similar to, but the delayed EC50 (123 ng/L) was markedly higher
than, that in the sensitivity, efficacy or no-hysteresis models (P < 0.001). The EC50 in the efficacy construct (10 ng/L) did not differ from that in the no-hysteresis construct (Table 1).

Exploratory regressions on age or BMI revealed a strongly negative correlation between age and downregulated ACTH efficacy in the efficacy-shift model (P = 0.0032, R2 = 0.30): Figure 6. There was a weak positive correlation between BMI and the hysteretic time shift in the same model (P = 0.037, R2 = 0.16).

Discussion

The stress pituitary-adrenal feedforward (stimulatory) interface was utilized as a prototypic dose-response connection in vivo to examine three complementary models of pulsatile dynamics of effector-response coupling using an archival data set (16; 30). The potency and sensitivity downregulation constructs both achieved a 40% reduction of mean model residual error compared with a non-hysteresis formulation, and the efficacy downregulation model a 20% reduction (P < 0.001 model contrasts). The mathematical framework so developed introduces a means to assess short-term downregulation or upregulation within individual effector-response pulse pairs. General applicability of the new analytical models was ensured by permitting either downregulation (inhibition) or upregulation (potentiation) of the dose-response process on a short time scale estimated simultaneously. According to this framework, estimated ACTH-cortisol dose-response downregulation shifts occur at a relatively consistent delay of 22 min after the onset of an adrenal cortisol secretory-burst response to a pituitary ACTH concentration pulse. The geometric mean delay of < 25 min (Table 1) indicates that the three models predict rather dramatic changes within the timespan of
one cortisol secretory episode.

The accompanying analyses unveil evidence for rapid effector-response adaptations within individual interpulse intervals in an unstressed *in vivo* physiological context. In contradistinction, prior studies have principally assessed pharmacological downregulation of hormonal systems (37; 39). Examples include pulses of gonadotropin-releasing hormone (GnRH), which stimulate secretion of luteinizing hormone (LH) pulses by the pituitary gland, and pulses of growth hormone (GH), which activate masculine profiles of body growth and JAK-STAT/5b signaling in the liver (38; 39). Pharmacological nonpulsatile infusions of GnRH and GH induce, respectively, hypogonadotropic hypogonadism and feminine patterns of somatic growth and hepatic gene expression (1; 9). Analogously, pharmacologically continuous ACTH delivery causes glucocorticoid-response desensitization in cultured adrenocortical cells (20; 26; 28; 33). Frequent sampling of cortisosterone secretion in the female rat has revealed further that stress either enhances or represses ongoing glucocorticoid secretion depending upon whether the stressor is imposed at the time of the rising or falling phase of the adrenal secretory burst (40). Although the precise mechanisms are not known, splanchnic innervation and diverse systemic and local factors (like ghrelin, glucagon, angiotensin II, interleukin 6, leptin, neurotensin, cholinergic and aminergic neurotransmitters, sex steroids, PGF-2 alpha, endothelin, galanin) can amplify or quench cortisol secretion by an ACTH stimulus (5; 8; 19; 21-25; 29; 31).

Physiological significance of adrenal-response downregulation in the human is inferable, inasmuch as a patient harboring a rare mutation of the cytoplasmic tail of the ACTH receptor manifested Cushing syndrome due to constitutively elevated cortisol
secretion. The latter was associated with impaired desensitization of the transfected mutant ACTH receptor (35). In the present analyses, age was associated with marked efficacy downregulation [Figure 6]. The mechanism mediating this effect is not known, and the finding should be confirmed in longitudinal studies.

An important conceptual implication of agonist-response adaptations on short time scales is that previously presumed stochastic variability may be further partitioned into an admixture of deterministic (dose-response) downregulation and stochastic (random effects on burst mass) processes. From a deterministic (causal) vantage, rapid effector-response adaptations are putatively mediated by way of membrane ion channels and/or short-lived phosphorylation and phosphatase reactions (6; 7; 12; 22; 25). To the degree that deterministic and stochastic mechanisms are quantifiable validly and reliably, their inclusion in models should make the overall evaluation of interlinked parameters more realistic and reproducible.

The generality of rapid pulsatile autoregulation of dose-response connections in other biological systems is not yet known. However, agonist-selective downregulation or upregulation within the interval of an individual pulse would offer a plausible explanation for attenuation of LH secretion by high-frequency GnRH pulses, and conversely for augmentation of hepatic IGF-I synthesis by high-frequency GH pulses (9). Such dynamics are not shared by FSH responses to rapid GnRH pulses (37) or muscle IGF-I responses to rapid GH pulses, thus suggesting selectivity of signaling-pathway and target-organ adaptations (11; 39).

**Perspectives**

To our knowledge, the ACTH-cortisol dose-response nexus represents the first
physiological linkage in which rapid reversible dynamics of an endogenous effector-response interface have been assessed noninvasively \textit{in vivo} in a quantitative model. If validated further by direct experimental interventions using physiological effector doses, this investigative strategy should also help parse the bases of recurrent cycles of desensitization and resensitization on short time scales in other contexts. Relevant applications could include both endocrine and nonendocrine control systems, such as GnRH-LH (39) and cardiovascular baroreceptor reflexes (4; 36).
Acknowledgments

We thank Donna Scott for support of manuscript preparation; Ashley Bryant for data analysis and graphics; the Mayo Immunochemical Laboratory for assay assistance; and the Mayo research nursing staff for implementing the protocol. Supported in part via the Center for Translational Science Activities (CTSA) Grant Number 1 UL 1 RR024150 from the National Center for Research Resources (Rockville, MD), and AG029362, AG019695 and DK073148 from the National Institutes of Health (Bethesda, MD).
Appendix: Cortisol Dose-Response to ACTH Feedforward with Hysteresis

To delineate the dose-response relationship of ACTH feedforward on (stimulation of) cortisol secretion, the modeling is performed in two stages. As a first stage, cortisol and ACTH are each individually modeled. Hence, the elimination and secretory structures for each hormone are estimated, without directly modeling the influence of the other. The statistical validity and accuracy of the methods have been established (38). For cortisol, the first stage recovers the cortisol secretion rate with its time-varying pattern. For ACTH, the first stage reconstructs a time-varying ACTH concentration (feedforward) profile. In the second stage, the estimated ACTH feedforward signal (input) and the cortisol secretion rate (output) are used to evaluate the dose-response relationship of ACTH concentrations on cortisol secretion: Figure 1. This two-phase strategy was used after exploratory modeling of cortisol made evident that, if one attempted to simultaneously estimate both cortisol half-lives of elimination and ACTH-modulated cortisol secretion, half-lives tended to be underestimated and cortisol-secretion rates overestimated.

Before describing the dose-response estimation, one needs to explicitly describe how the estimated ACTH feedforward signal and cortisol secretion rate were obtained. The modeling of the concentrations for both ACTH and cortisol each involve three elements: their respective pulse times, their secretion rates and the fast and slow rates of elimination of each. Let the putative pulse times for cortisol and ACTH, respectively, be denoted as: $T_{C}^{(1)}, T_{C}^{(2)}, \ldots T_{C}^{(m_{C})}$ and $T_{A}^{(1)}, T_{A}^{(2)}, \ldots T_{A}^{(m_{A})}$, where the numbers of pulses are $m_{C}$ and $m_{A}$. The pulse times and number of pulses are to be estimated. Also, in previous modeling it was shown that there are day-night differences in the waveform of secretion.
for both cortisol and ACTH (17). A unit area-normalized rate of secretion over time ( waveform) is described by a 3-parameter generalized Gamma density, one for day (D) and one for night (N). The day-night separation is estimated, with day containing the interval: $[\phi_1, \phi_2]$. For $r=\text{cortisol (C), ACTH (A)}$, the waveforms are:

$$
\psi_r(s) \propto s^{\beta_r(D) - 1} e^{-s/\beta_r(D)} + (s/\beta_r(N) - 1) e^{-s/\beta_r(N)}, \quad \phi_{r,1} \leq T_r^{(k)} \leq \phi_{r,2}, \quad r = \text{C,A} \quad (1)
$$

The secretion rates are then given as follows. Each secretory-burst mass is described as a linear function of the preceding interpulse interval $(T_r^{(k)} - T_r^{(k-1)})$ plus a random effect $(A)$. The latter allows for variation in successive burst size, which is not explicitly modeled by a linear function:

$$
Z_C(t) = \beta_C + \sum_{r=1}^{n} (\eta_{0,C} + \eta_{1,C} \times (T_C^{(k)} - T_C^{(k-1)})) + A_C^{(k)} \psi_C(t - T_C^{(k)}) \quad (2)
$$

$$
Z_A(t) = \beta_A + \sum_{r=1}^{n} (\eta_{0,A} + \eta_{1,A} \times (T_A^{(k)} - T_A^{(k-1)})) + A_A^{(k)} \psi_A(t - T_A^{(k)}).
$$

If the fast and slow rates of elimination, for $r=\text{C,A}$, are denoted as: $\alpha_r^{(1)}$ and $\alpha_r^{(2)}$, with fractions: $a_r$ and $1-a_r$, then the resulting concentrations are:

$$
X_C(t) = (a_c e^{-\alpha_c^{(1)}t} + (1-a_c) e^{-\alpha_c^{(2)}t}) X_C(0) + \int_0^t (a_c e^{-\alpha_c^{(1)}(t-r)} + (1-a_c) e^{-\alpha_c^{(2)}(t-r)})) \times Z_C(r) dr \quad (3)
$$

$$
X_A(t) = (a_a e^{-\alpha_a^{(1)}t} + (1-a_a) e^{-\alpha_a^{(2)}t}) X_A(0) + \int_0^t (a_a e^{-\alpha_a^{(1)}(t-r)} + (1-a_a) e^{-\alpha_a^{(2)}(t-r)})) \times Z_A(r) dr \quad (4)
$$

Finally, what are then observed are the concentrations with measurement error:

$$
Y_{r,i} = X_r(t_i) + \epsilon_r(i), \quad i=1,\ldots,n, \quad r=\text{C, A} \quad (5)
$$

Let $\theta_r$, for $r=\text{C,A}$, denote parameters for the cortisol and the ACTH models.
Parameter estimation then proceeds by penalized maximum-likelihood estimation (MLE), where the penalization is on the number of pulse times, \( m_c \) for cortisol and \( m_A \) for ACTH. Once the pulse times and the parameter estimates \( \hat{\theta}_r \) are obtained, the two secretion rates can be estimated as conditional expectations evaluated at their MLE \( \hat{\theta}_r \) (for \( r=C,A \)):

\[
\hat{Z}_r(i = 1,\ldots,n) = E_{\hat{\theta}_r} [Z_r(t_i), i = 1,\ldots,n \mid Y_r, i = 1,\ldots,n], r = C, A
\]  (6)

This calculation involves the conditional expectations of the random effects, conditioned on the observed concentrations. Once these are obtained, one can then calculate the model fits, viz., the predicted concentrations. That is the fits are obtained by a convolution (expressions (3)-(4)) using the estimated secretion rates (expression (6)) and the estimated biexponential kinetics. The result is the predicted (reconvolved) concentrations:

\[
\hat{Y}_r(i = 1,\ldots,n), r = C, A.
\]  (7)

**Figure 2** highlights the above step for an illustrative subject: (left), cortisol and ACTH concentrations (top and bottom), their corresponding predicted concentrations (fit) and their estimated pulse times; (middle), the estimated secretion rates for cortisol and ACTH; and, (right), the estimated day (D) and night (N) waveforms for cortisol and ACTH.

The results of the above (first step) are: (i) reconvolved ACTH concentrations: \( \hat{Y}_A(i = 1,\ldots,n) \), expression (7), which constitute the ACTH feedforward signal on cortisol secretion; and (ii) estimated cortisol secretion rates: \( \hat{Z}_C(i = 1,\ldots,n) \), expression (6).

These are the core elements used for the second step: estimation of the dose-response
relationship of ACTH concentrations on cortisol secretion. Two important issues arise in this formulation. First, there is a time delay in the effect of ACTH concentrations on cortisol secretion (Figures 2 and 3, top row), which may vary in time. Secondly, desensitization (tachyphylaxis) could result in a changing nature to any such dose-response relationship. Both of these issues must be resolved if one hopes to extract the unobserved dose-response relationship.

To accommodate the potentially time-varying nature of the time delay of ACTH on cortisol, we proceed as follows: for each cortisol pulse time ($T_{C}^{(k)}$), the nearest ACTH pulse ($T_{A}^{(j)}$) within a [-40, 10] min window was identified, if one existed. (The possibility that an ACTH pulse slightly succeeded a cortisol pulse was allowed due to adrenal splanchnic innervation.) The ACTH pulse was then shifted so that its onset aligned with the onset of the cortisol pulse. Figure 3 (bottom row) displays the ACTH fit $\hat{Y}_{A,j}$ (solid line) and the ACTH feedforward signal $F_{A}(i)$ (dashed line), which result from local pulse-time alignments.

The ACTH feedforward signal $F_{A}(i)$ is then assumed to feed through an estimable four-parameter logistic dose-response function to produce the deconvolution-calculated cortisol secretion rate, $Z_{C}(t)$: Figure 3 (top row). Dose-response parameter estimation proceeds by comparison of the latter with the recursively estimated cortisol secretion rate $\hat{Z}_{C,i}$ ($i=1,...,n$), assumed to be $Z_{C}(t_{i})$ + error. Random effects (A’s) in efficacy are included in order to accommodate pulse-by-pulse variation.

In order to allow for desensitization of a cortisol response, an allowance for change in the response mechanism, specifically a possible mid-pulse shift in the dose response, is included (Figure 3, middle row). That is, for an amount of time $M_{AssC}$ (a parameter to
be estimated) following the onset of a cortisol pulse, one dose-response curve is followed; after that time, there is a shift to a new dose-response curve to which the ACTH feedforward signal $F_A(t)$ also applies. Specifically, three models of the dose-response change are considered. They represent, respectively, the change in dose-response via a shift in potency, a shift in sensitivity, and a shift in efficacy.

Model 1: Half-Maximally Effective Stimulus Concentration (ACTH Potency):

$$
\hat{Z}_C(t_i) = \begin{cases}
\eta_0 + \frac{\eta_i + A_{AonC}^{(k)}}{1 + \exp\{- (\eta_i^{UP} + \eta_i \times F_A(t_i))\} }, & T_C^{(i)} \leq t_i < T_C^{(i) + M_{AonC}} \\
\eta_0 + \frac{\eta_i + A_{AonC}^{(k)}}{1 + \exp\{- (\eta_i^{DOWN} + \eta_i \times F_A(t_i))\} }, & T_C^{(i) + M_{AonC}} \leq t_i < T_C^{(i+1)} \\
\end{cases} + \epsilon_i, i = 1, \ldots, n
$$

Model 2: Dose-Response Slope (Adrenal Sensitivity):

$$
\hat{Z}_C(t_i) = \begin{cases}
\eta_0 + \frac{\eta_i + A_{AonC}^{(k)}}{1 + \exp\{- (\eta_i^{UP} + \eta_i \times F_A(t_i))\} }, & T_C^{(i)} \leq t_i < T_C^{(i) + M_{AonC}} \\
\eta_0 + \frac{\eta_i + A_{AonC}^{(k)}}{1 + \exp\{- (\eta_i^{DOWN} + \eta_i \times F_A(t_i))\} }, & T_C^{(i) + M_{AonC}} \leq t_i < T_C^{(i+1)} \\
\end{cases} + \epsilon_i, i = 1, \ldots, n
$$

Model 3: Asymptotic Maximum (ACTH Efficacy):

$$
\hat{Z}_C(t_i) = \begin{cases}
\eta_0 + \frac{\eta_i^{UP} + A_{AonC}^{(k)}}{1 + \exp\{- (\eta_i + \eta_i \times F_A(t_i))\} }, & T_C^{(i)} \leq t_i < T_C^{(i) + M_{AonC}} \\
\eta_0 + \frac{\eta_i^{DOWN} + A_{AonC}^{(k)}}{1 + \exp\{- (\eta_i + \eta_i \times F_A(t_i))\} }, & T_C^{(i) + M_{AonC}} \leq t_i < T_C^{(i+1)} \\
\end{cases} + \epsilon_i, i = 1, \ldots, n
$$

In Figure 3, the three columns (left, middle, right) represent the applications of the above three models for the same subject as in Figure 2.
Maximum-likelihood estimation (MLE) of the dose-response parameters is performed. **Figure 3** (*middle row*) displays results for the present subject. The *solid line* (logistic curve) denotes the estimated initial mean dose-response, and the *dashed line*, the ending mean dose-response (after time $M_{\text{donC}}$ from the cortisol pulse onset) feedforward function. Interrupted fine lines denote the pulse-by-pulse reconstruction of dose-response curves, each with the allowed random effect in efficacy. The model thus defines a change in the cortisol response to ACTH, in which initial ACTH concentrations appear to proceed up one curve, but return as the cortisol pulse diminishes via a different curve.
References


35. Swords FM, Baig A, Malchoff DM, Malchoff CD, Thorner MO, King PJ, Hunyady L and Clark AJ. Impaired desensitization of a mutant


Legends

**Figure 1.** Schema of four complementary ACTH-cortisol dose-response model structures, one without (*top middle*) and three with allowable hysteresis-like autoregulation of pulsatile feedforward (stimulatory) potency, sensitivity or efficacy (*bottom, left to right*).

**Figure 2.** Illustrative deconvolution of cortisol (*top*) and ACTH (*bottom*) concentration-time series (*left*) with resultant secretion profiles (*middle*) given mathematical allowance for two possible secretory-burst waveforms in each profile (*right*). Asterisks on the x axis signify pulse onsets. Open diamonds denote the times of the changepoints of the inferred waveform.

**Figure 3.** Illustrative dose-response estimates in one individual comprising ACTH concentration-dependent drive of cortisol secretion according to three models of allowable hysteretic shifts in potency (*left*), sensitivity (*middle*) and efficacy (*right*) in one subject [same subject as in Figure 2]. The *top* row gives deconvolution-calculated cortisol secretion (continuous lines) and dose-response predicted cortisol secretion (interrupted lines); the *middle* row the time-shifted reconvolved ACTH concentration profile (interrupted curve with asterisks for pulse locations) and unshifted cortisol secretion profile (continuous line with diamonds for pulse locations); the *bottom* row mean dose-response (*solid curve* for initial and *interrupted curve* for downregulated) estimates and random effects on efficacy (*dotted*). Light interrupted lines define pulse-by-pulse random effects on efficacy. Time shifts are in min. Data for all 28 subjects are given in **Table 1**.

**Figure 4.** Outcomes of estimated hystereses models (unequal initial (I) and delayed (D)
dose-response parameters) [bottommost terms] comprising allowable shifts in any one of potency, sensitivity or efficacy after a finite and estimable time delay from the onset of a cortisol secretory burst. Data in each of the 3 panels are the geometric mean ± SD of the indicated parameter stated above the panel. Results are from 28 healthy adults. The solid and interrupted horizontal lines give the mean ± SD of the base (no hysteresis) model. Unshared alphabetic superscripts define differing geometric mean using the post hoc Tukey’s test.

**Figure 5.** Comparison among downregulation models of estimated ACTH EC$_{50}$ values (estimated effector concentration stimulating a one-half maximal cortisol secretory response). The interrupted lines give the geometric mean ± SD for the base model without hysteresis (N = 28 adults). EC$_{50}$ is defined by -potency/sensitivity in the dose-response model. Accordingly, two EC$_{50}$ values are estimable each for the hysteretic potency and sensitivity models, one for initiation and one for downregulation of cortisol pulses, and a single EC$_{50}$ for efficacy hysteresis.

**Figure 6.** Regression on age of downregulated ACTH efficacy (delayed efficacy) in 28 healthy adults. The single boxed value (marked 20.5) is a statistical outlier at P < 0.001 by Studentized residuals. Data in men (closed circles) and women (open circles) were combined.
Table 1. Comparisons of ACTH-Cortisol Hystereses Models.

<table>
<thead>
<tr>
<th>N = 28</th>
<th>Model</th>
<th>Potency Model</th>
<th>Sensitivity Model</th>
<th>Efficacy Model</th>
<th>ANCOVA</th>
<th></th>
<th>Kruskal-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Hysteresis</td>
<td>9.4 (4.1 - 21)(^A)</td>
<td>5.5 (2.2 - 14)(^B)</td>
<td>5.2 (2.1 - 13)(^B)</td>
<td>7.2 (2.9 - 18)(^C)</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>(\delta_e)</td>
<td>1.9 (0.01 - 365)(^A)</td>
<td>14 (2.9 - 71)(^B)</td>
<td>11 (2.9 - 46)(^B)</td>
<td>1.1 (0.02 - 55)(^A)</td>
<td>&lt; 0.001</td>
<td>0.006</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Time shift</td>
<td>NA</td>
<td>22 (12 - 39)</td>
<td>22 (13 - 39)</td>
<td>22 (12 - 39)</td>
<td>0.962(^*)</td>
<td>-------</td>
<td>0.878</td>
</tr>
<tr>
<td>Basal</td>
<td>1.3 (0.12 - 14)(^A)</td>
<td>1.1 (0.25 - 5.1)(^A)</td>
<td>1.1 (0.28 - 4.8)(^A)</td>
<td>0.20 (0.00 - 10)(^B)</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Potency(1)</td>
<td>-7.6 (-2.2 to -26)(^A)</td>
<td>-2.1 (-0.51 to -8.9)(^B)</td>
<td>-8.7 (-3.5 to -21)(^A)</td>
<td>-3.1 (-0.81 to -12)(^B)</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sensitivity(1)</td>
<td>0.47 (0.10 - 2.25)(^A)</td>
<td>0.23 (0.15 - 0.36)(^B)</td>
<td>0.97 (0.27 - 3.5)(^C)</td>
<td>0.30 (0.04 - 2.3)(^AB)</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Efficacy(1)</td>
<td>11 (1.8 - 67)(^A)</td>
<td>19 (6.1 - 58)(^B)</td>
<td>15 (5.5 - 43)(^AB)</td>
<td>22 (9.2 - 51)(^B)</td>
<td>0.001</td>
<td>0.076</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Potency(2)</td>
<td>NA</td>
<td>-12 (-7.4 to -21)(^A)</td>
<td>-8.7 (-3.5 to -21)(^B)</td>
<td>-3.1 (-0.81 to -12)(^C)</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sensitivity(2)</td>
<td>NA</td>
<td>0.23 (0.15 - 0.36)(^A)</td>
<td>0.071 (0.01 - 0.68)(^B)</td>
<td>0.30 (0.04 - 2.3)(^A)</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Efficacy(2)</td>
<td>NA</td>
<td>19 (6.1 - 58)(^A)</td>
<td>15 (5.5 - 43)(^A)</td>
<td>1.3 (0.05 - 30)(^B)</td>
<td>&lt; 0.001</td>
<td>0.033</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>EC(_{50})(1)</td>
<td>16 (2.1 - 127)(^A)</td>
<td>9.4 (2.3 - 39)(^B)</td>
<td>8.9 (3.2 - 25)(^B)</td>
<td>10 (0.93 - 114)(^B)</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>EC$_{50}(2)$</td>
<td>NA</td>
<td>54 (27 - 111)$^A$</td>
<td>123 (10 - 1457)$^B$</td>
<td>10 (0.93 - 114)$^C$</td>
<td>&lt; 0.001</td>
<td>0.039</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Data are the geometric mean (95% confidence interval). EC$_{50}$ = potency/sensitivity. Cortisol secretion rates (basal, efficacy) are nmol/L/min. ACTH concentration (EC$_{50}$) are ng/L. NA = Not applicable. *ANOVA was performed on time-shift data, since there was no covariate.

Parenthetical 1 and 2 denote onset and offset (initiation and delayed-recovery) phases of cortisol secretory bursts.
Figure 1.

Three Complementary Models of Hysteresis

- **Random effects (\( \delta_A \))**
  - Base Model

- **ACTH Concentration**
  - Potency Model
  - Sensitivity Model
  - Efficacy Model

- **Cortisol Secretion**
  - ACTH Con
    - Shift

- **Cort Sec**
  - ACTH Con
    - Shift

X:\SEC\Data\Roelfsema\ACTH-Cortisol\From Dan Keenan Jan13 2010\Hysteresis Models.ppt

Z:\SHARED\Manuscripts\2010 [681] AJP Fdfwd Model\Revision\Figures.doc
Figure 2.

Cortisol and ACTH Concentrations and Secretion Rates

Subject 11
Figure 3.

Three Hystereses Models: ACTH’s Drive of Cortisol Secretion

Potency Model

Sensitivity Model

Efficacy Model

Cortisol Sec (nmol/L/min)

ACTH Con (ng/L)

Shift Time: 28.8

Shift Time: 29.4

Subject 11

ACTH Concentration (ng/L)

Clock Time (hr)

0900 1700 0100 0900 0900 1700 0100 0900 0900 1700 0100 0900

Z:\SHARED\Manuscripts\2010 [681] AJP Fdfwd Model\Revision\Figures.doc
Figure 4.

Downregulation Models of ACTH-Cortisol Drive

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sensitivity</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Potency</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Efficacy</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

A
B
C

X:\SEC\Data\Roelfsema\ACTH-Cortisol\From Dan Keenan Jan13 2010\Figs for 2010 ENDO Abstract\Figure 4.ppt

Z:\SHARED\Manuscripts\2010 [681] AJP Fdfwd Model\Revision\Figures.doc
Figure 5.

EC₅₀ for Hysteretic ACTH-Cortisol Feedforward Model

N = 28 adults
Figure 6.

Impact of Age on Downregulated ACTH-Cortisol Efficacy

Efficacy Hysteresis Model

\[
P = 0.0032 \\
R^2 = 0.30 \\
slope = -0.080 \pm 0.025
\]

Men (N = 13) 
Women (N = 15)