Dose-response downregulation within the span of single interpulse intervals

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1Department of Statistics, University of Virginia, Charlottesville, Virginia; 2Department of Endocrine and Metabolic Diseases, Leiden University Medical Center, Leiden, The Netherlands; and 3Endocrine Research Unit, Mayo School of Graduate Medical Education, Center for Translational Science Activities, Mayo Clinic, Rochester, Minnesota

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Keenan DM, Roelfsema F, Veldhuis JD. Dose-response downregulation within the span of single interpulse intervals. Am J Physiol Regul Integr Comp Physiol 299: R11–R18, 2010. First published April 21, 2010; doi:10.1152/ajpregu.00201.2010.—Pituitary 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 h in the unstressed state (8,120 measurements). ACTH concentration-cortisol secretion dose-response functions were then estimated in each subject without hysteresis (base model) and with allowances for possible hysteresis in 2 ACTH potency, 3 adrenal sensitivity, and 4) 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, i.e., grand mean (95% confidence interval) 22 (12–39) min after the onset of a cortisol secretory burst. Half-maximally effective ACTH concentrations (EC50) differed before and after hysteretic inflection within individual pulses: 1) 9.4 and 54 ng/l in the potency model (P < 0.001) and 2) 8.9 and 123 ng/l in the sensitivity model (P < 0.001) compared with 16 ng/l in 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−1·min cortisol secretion−1, 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 in vivo. This construct should have utility in parsing mechanisms of physiological regulation in other integrative systems.

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Methods

METHODS

Human subjects. Conventional cross-correlation, approximate entropy, and dose-response evaluation of these ACTH and cortisol time series have been reported (16, 30). The present analyses of these archival data do not overlap with earlier outcomes or methods. Briefly, 28 volunteers (13 men and 15 women) provided informed consent to participate in the study, which was approved by the Leiden University Institutional Review Board. The age range of the subjects was 30–77 yr, and their body mass index (BMI) was 18–30 kg/m². They 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).

Subjects were admitted to the Leiden Study Unit for overnight adaptation before undergoing frequent blood sampling beginning at 0900 on the next day (30). Blood was collected every 10 min for 24 h into ice-cold siliconized tubes containing EDTA (ACTH) or heparin (cortisol), centrifuged in the cold, and frozen within 30 min of collection, as described elsewhere (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 (2-parameter Weibull renewal process); admixed basal and pulsatile secretions; hormone- and subject-specific biexponential elimination kinetics; a flexible (3-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-h 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: 1) efficacy (asymptotically projected maximal ACTH-stimulated cortisol secretion rate, mmol·l⁻¹·min⁻¹); 2) potency [an exponential measure related inversely to the ACTH concentration driving half-maximal ACTH secretion (EC50)]; 3) sensitivity (maximal positive slope of the ACTH-cortisol dose-response relationship); and 4) basal (nonpulsatile) cortisol secretion (15, 16). The innovation is to extend this structure to allow for three possible types of dose-response hysteresis 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 potency, sensitivity, or efficacy (Fig. 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; after this time, it operates 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 designated δs. Residual model error (δe) is estimated concurrently from comparison of model-predicted and deconvolution-estimated (cortisol) sample secretory bursts.

Statistical analyses. One-way analysis of covariance (ANCOVA) with three categorical factors (the 3 hysteresis models) was the primary statistical model, wherein the no-hysteresis (base) model output served as the covariate. Data were first transformed using the natural logarithm. Thus, parameters of the model are given in Table 1 as the geometric mean (95% confidence interval) and in Figs. 4 and 5 as the same ± SD. Other data are reported as arithmetic means ± SE or medians (ranges). Post hoc Tukey’s honestly significantly difference test was used for multiple comparisons at experiment-wise protected P ≤ 0.05. ANCOVA results were confirmed by the Kruskal-Wallis nonparametric test. Student’s two-tailed, unpaired, common-variance t-statistic was used to compare dose-response parameters by sex 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 three dynamic models of effector dose-secretory responses proposed (see METHODS) and examined here. The three separate hysteresis 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 nighttime, thus constituting a dual-waveform model of secretion. Two change-point times were estimated to demarcate onset of the day- and nighttime waveforms within each 24-h pulse train (as discussed fully in Ref. 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 four-parameter logistic dose-response function without hysteresis (base model), a five-parameter hysteretic potency model (sep-
arate ACTH potencies for initial and delayed segments of cortisol secretory bursts), and the latter structure, but embodying two sensitivities or two efficacies (Fig. 3). ANCOVA of model residual errors (with the no-hysteresis model residual error used as the covariate) revealed a descending order of goodness of fit in the set of 28 subjects as follows: 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 the potency and sensitivity models and 20% lower in the efficacy model than in the nonhysteresis (base) model. For the three hysteresis models, initial and delayed sensitivity differed by 13.7-fold (sensitivity model), potency differed by 5.6-fold (potency model), and efficacy differed 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. These comparisons are summarized in Table 1 ($\delta_c$ and $\delta_A$). In contradistinction, mean time delays for hysteresis were comparable among the three dose-response

Fig. 2. Deconvolution prediction (orange) of measured (blue) cortisol (top) and ACTH (bottom) concentration-time series (left), with resultant secretion profiles (middle), given mathematical allowance for 2 possible secretory-burst waveforms in each profile (right). *On x-axis, pulse onset; ⊙, times of change points of inferred waveform.

Fig. 3. Dose-response estimates in 1 subject (same subject and protocol used in Fig. 2) comprising ACTH concentration-dependent drive of cortisol secretion according to 3 models of allowable hysteretic shifts in potency, sensitivity, and efficacy. Top: deconvolution-calculated cortisol secretion (solid blue lines) and dose-response predicted cortisol secretion (dashed orange lines); middle: time-shifted reconverted ACTH concentration profile (dashed curve with asterisks for pulse locations) and unshifted cortisol secretion profile (solid line with diamonds for pulse locations); bottom: mean dose-response [initial (solid curve) and downregulated (dashed curve)] 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.
models at 22 (12–39) min [geometric mean (95% confidence interval), $P = 0.962$ by ANCOVA, $P = 0.878$ by Kruskal-Wallis test; Table 1, time shift].

Parameter estimates in the three hysteresis models were compared by ANCOVA. Mean basal (nonpulsatile) cortisol secretion was about sixfold lower in the efficacy hysteresis model ($P < 0.001$) than in the potency, efficacy, or no-hysteresis model (Table 1, basal; $P < 0.001$). The exponential parameters for potency and sensitivity and the numerator term for efficacy differed by hysteresis model (each $P < 0.001$; Fig. 4). In particular, mean absolute (unsigned) potency was about threefold higher in the no-hysteresis model ($P < 0.001$) than in the other three models (Table 1, basal; $P < 0.001$). The exponential parameters for potency and sensitivity compared with 24 ± 2 ng/l (arithmetic mean) in an earlier estimate using a nonhysteris dose-response model (16). In the potency hysteresis construct, initial and delayed EC$_{50}$ values were 9.4 and 54 ng/l ($P < 0.001$ by Tukey’s test). The initial EC$_{50}$ (8.9 ng/l) in the sensitivity model was similar to, but the delayed EC$_{50}$ (123 ng/l) was markedly higher than, that in the potency, efficacy, or no-hysteresis model ($P < 0.001$). The EC$_{50}$ in the efficacy construct (10 ng/l) did not differ from that in the no-hysteresis construct (Table 1).

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Inasmuch as the EC$_{50}$ for ACTH drive is defined numerically as –potency/sensitivity, EC$_{50}$ provides an ensemble measure of submaximal stimulatory effects. Figure 5 depicts EC$_{50}$ values (ng/l ACTH concentrations) associated with each of the three hysteresis models compared with the no-hysteresis base model. The base model, with two allowed secretory-burst shapes, yielded a geometric mean EC$_{50}$ of 16 ng/l (and an arithmetic mean ± SE of 23 ± 3.3 ng/l) compared with 24 ± 2.1 ng/l (arithmetic mean) in an earlier estimate using a nonhysteris dose-response model (16). In the potency hysteresis construct, initial and delayed EC$_{50}$ values were 9.4 and 54 ng/l ($P < 0.001$ by Tukey’s test). The initial EC$_{50}$ (8.9 ng/l) in the sensitivity model was similar to, but the delayed EC$_{50}$ (123 ng/l) was markedly higher than, that in the potency, efficacy, or no-hysteresis model ($P < 0.001$). The EC$_{50}$ in the efficacy construct (10 ng/l) did not differ from that in the no-hysteresis construct (Table 1).

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#### Table 1. Comparisons of ACTH-cortisol hysteresis models

<table>
<thead>
<tr>
<th>Model</th>
<th>No-Hysteresis</th>
<th>Potency</th>
<th>Sensitivity</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>δ$_{A}$ 9.4 (4.1–21)*</td>
<td>5.5 (2.2–14)†</td>
<td>5.2 (2.1–13)†</td>
<td>7.2 (2.9–18)‡</td>
</tr>
<tr>
<td></td>
<td>δ$_{B}$ 1.9 (0.01–36)*</td>
<td>14 (2.9–71)†</td>
<td>11 (2.9–46)†</td>
<td>1.1 (0.02–55)*</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>
</tr>
<tr>
<td>Basal</td>
<td>1.3 (0.12–14)*</td>
<td>1.1 (0.25–5.1)*</td>
<td>1.1 (0.28–4.8)*</td>
<td>0.20 (0.00–10)†</td>
</tr>
<tr>
<td>Potency (1)</td>
<td>−7.6 (−2.2 to −26)*</td>
<td>−2.1 (−0.51 to −8.9)†</td>
<td>−8.7 (−3.5 to −21)‡</td>
<td>−3.1 (−0.81 to −12)‡</td>
</tr>
<tr>
<td>Sensitivity (1)</td>
<td>0.47 (0.10–2.25)*</td>
<td>0.23 (0.15–0.36)†</td>
<td>0.97 (0.27–3.5)‡</td>
<td>0.30 (0.04–2.3)*‡</td>
</tr>
<tr>
<td>Efficacy (1)</td>
<td>11 (1.8–67)*</td>
<td>19 (6.1–58)†</td>
<td>15 (5.5–43)‡</td>
<td>22 (9.2–51)†</td>
</tr>
<tr>
<td>Potency (2)</td>
<td>NA</td>
<td>−12 (−7.4 to −21)‡</td>
<td>−8.7 (−3.5 to −21)‡</td>
<td>−3.1 (−0.81 to −12)‡</td>
</tr>
<tr>
<td>Sensitivity (2)</td>
<td>NA</td>
<td>0.23 (0.15–0.36)*</td>
<td>0.071 (0.01–0.68)†</td>
<td>0.30 (0.04–2.3)*‡</td>
</tr>
<tr>
<td>Efficacy (2)</td>
<td>NA</td>
<td>19 (6.1–58)*</td>
<td>15 (5.5–43)*</td>
<td>1.3 (0.05–30)†</td>
</tr>
<tr>
<td>EC$_{50}$ (1)</td>
<td>16 (2.1–127)*</td>
<td>9.4 (2.3–39)‡</td>
<td>8.9 (3.2–25)‡</td>
<td>10 (0.93–114)‡</td>
</tr>
<tr>
<td>EC$_{50}$ (2)</td>
<td>NA</td>
<td>54 (27–111)*</td>
<td>123 (10–1457)‡</td>
<td>10 (0.93–114)‡</td>
</tr>
</tbody>
</table>

Values are geometric means (95% confidence intervals); $n = 28$. EC$_{50}$, –potency/sensitivity. Cortisol secretion rates (basal, efficacy) are nmol·l$^{-1}$·min$^{-1}$. ACTH concentrations (EC$_{50}$) are ng/l. ANCOVA, analysis of covariance; 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. Different symbols (*, †, ‡) define differing geometric mean (by post hoc Tukey’s test).

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Fig. 4. Outcomes of estimated hysteresis models [unequal initial (I) and delayed (D) dose-response parameters] comprising allowable shifts in potency (Pot), sensitivity (Sen), or efficacy (Eff) after a finite and estimable time delay from onset of a cortisol secretory burst. Data are geometric means ± SD from 28 healthy adults. Solid and dashed horizontal lines, mean ± SD of the base (no-hysteresis) model. Unshared alphabetic superscripts (A, B, and C) define differing geometric mean (by post hoc Tukey’s test).

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AJP-Regul Integr Comp Physiol • VOL 299 • JULY 2010 • www.ajpregu.org
hysteretic potency and sensitivity models (1 for initiation and 1 for downregulation which activate masculine profiles of body growth and JAK-by the pituitary gland, and pulses of growth hormone (GH), which stimulate secretion of luteinizing hormone (LH) pulses include pulses of gonadotropin-releasing hormone (GnRH), of effector-response couple using an archival data set (16, 30). To the degree that 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 known. However, agonist-selective downregulation or upregulation within the interval of an individual pulse would offer a plausible 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 corticosterone secretion in the female rat revealed further that stress enhances or represses ongoing glucocorticoid secretion, depending on whether the stressor is imposed at 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 (e.g., ghrelin, glucagon, angiotensin II, interleukin 6, leptin, neurotensin, cholinergic and aminergic neurotransmitters, sex steroids, PGF2α, endothelin, and 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’s 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 (Fig. 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 accompanying analyses unveil evidence for rapid effector-response adaptations within individual interpulse intervals in an unstressed in vivo physiological context. In contrast distinction, prior studies have principally assessed pharmacological downregulation of hormone 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
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 achieved a 40% reduction of mean model residual error compared with a nonhysteretic formulation, and the efficacy downregulation model achieved 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 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 time span of one cortisol secretory episode.

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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; Fig. 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 achieved a 40% reduction of mean model residual error compared with a nonhysteretic formulation, and the efficacy downregulation model achieved 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 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 time span of one cortisol secretory episode.

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sible explanation for attenuation of LH secretion by high-frequency GnRH pulses and, conversely, for augmentation of hepatic insulin-like growth factor I synthesis by high-frequency GH pulses (9). Such dynamics are not shared by follicle-stimulating hormone responses to rapid GnRH pulses (37) or muscle insulin-like growth factor I responses to rapid GH pulses, thus suggesting selectivity of signaling pathway and target organ adaptations (11, 39).

**Perspectives and Significance**

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 in vivo in a quantitative model. If validated further by direct experimental interventions using physiological effector doses, this investigative strategy should also help parsel the bases of recurrent cycles of desensitization and resensitization on short time scales in other contexts. Relevant applications could include endocrine and nonendocrine control systems, such as GnRH-LH (39) and cardiovascular baroreceptor reflexes (4, 36).

**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 direct modeling of 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 (feedback 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 to cortisol secretion (Fig. 1). This two-phase strategy was used after exploratory modeling of cortisol showed that if one were to attempt to simultaneously estimate cortisol half-lives of elimination and ACTH-modulated cortisol secretion, half-lives would tend to be underestimated and cortisol secretion rates would tend to be 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 ACTH and cortisol 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 follows: $T_C^{(1)}, T_C^{(2)}, \ldots, T_C^{(m_C)}$ 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, previous estimates of cortisol secretion rates for cortisol and ACTH, and the estimated daytime and nighttime waveforms are as follows

$$Z_C(t) = \beta_C + \sum_{T_C^{(i)}} \left\{ n_{0C} + n_{1C} \times \left[ T_C^{(i)} - T_C^{(-1)} \right] + A_C^{(i)} \right\} \Psi_C \left[ t - T_C^{(i)} \right]$$

$$Z_A(t) = \beta_A + \sum_{T_A^{(i)}} \left\{ n_{0A} + n_{1A} \times \left[ T_A^{(i)} - T_A^{(-1)} \right] + A_A^{(i)} \right\} \Psi_A \left[ t - T_A^{(i)} \right]$$

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

$$X_C(t) = \left[ a_C e^{-\alpha^{(1)}_r t} + (1 - a_C) e^{-\alpha^{(2)}_r t} \right] X_C(0) + \int_0^t \left[ a_C e^{-\alpha^{(1)}_r (t-r)} + (1 - a_C) e^{-\alpha^{(2)}_r (t-r)} \right] Z_A(r) dr$$

$$X_A(t) = \left[ a_A e^{-\alpha^{(1)}_r t} + (1 - a_A) e^{-\alpha^{(2)}_r t} \right] X_A(0) + \int_0^t \left[ a_A e^{-\alpha^{(1)}_r (t-r)} + (1 - a_A) e^{-\alpha^{(2)}_r (t-r)} \right] Z_C(r) dr$$

Finally, the concentrations with measurement error are observed

$$Y_{c,i} = X_C(t_i) + e_{c,i} \quad i = 1, \ldots, n \quad r = C, A$$

Let $\theta_{r,i}$ for $r = C, A$, denote parameters for the cortisol and ACTH models. Parameter estimation then proceeds by penalized maximum-likelihood estimation, 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 $\theta_r$ are obtained, the two secretion rates can be estimated as conditional expectations evaluated at their maximum-likelihood estimation $\hat{\theta}_r$ (for $r = C, A$)

$$\hat{Z}_r(i = 1, \ldots, n) = E_{\hat{\theta}_r} \left[ Z_r(t), i = 1, \ldots, n \right] Y_{r,i}, i = 1, \ldots, n$$

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

$$\hat{Y}_{r,i} = \hat{Z}_r(i = 1, \ldots, n \quad r = C, A$$

Figure 2 highlights the above-described step for an illustrative subject: cortisol and ACTH concentrations, their corresponding predicted concentrations (fit), and their estimated pulse times, the estimated secretion rates for cortisol and ACTH, and the estimated daytime and nighttime waveforms for cortisol and ACTH.

The results of the first step (see above) are as follows: 1) reconvolved ACTH concentrations, $\hat{Y}_A, i = 1, \ldots, n$ (Eq. 7), which constitute the ACTH feedforward signal on cortisol secretion, and 2) estimated cortisol secretion rates, $\hat{Z}_C, i = 1, \ldots, n$ (Eq. 6). These are the core elements used for the second step, i.e., estimation of the dose-response relationship of ACTH concentrations on cortisol secretion. Two important issues arise in this formulation: 1) There is a time delay in the effect of ACTH concentrations on cortisol secretion (Figs. 2 and 3, top), which may vary in time, and 2) desensitization (tachyphylaxis) could result in a change in the nature of 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 \([T_k^{k+1}]\), the nearest ACTH pulse \([T_k^{k+1}]\) within a \([-40, 10]\) min window was identified, if one existed. (The possibility that an ACTH pulse slightly succeeded a cortisol pulse was allowed because of adrenal splanchnic innervation.) The ACTH pulse was then shifted, so that its onset aligned with the onset of the cortisol pulse. Figure 3 (bottom) displays the ACTH fit \(\hat{Y}_A(t)i\) (solid line) and the ACTH feedforward signal \(F_A(t)\) (dotted line) that result from local pulse-time alignments.

The ACTH feedforward signal \(F_A(t)\) is then assumed to feed through an estimable four-parameter logistic dose-response function to produce the deconvolution-calculated cortisol secretion rate, \(Z_C(t)\) (Fig. 3, top). Dose-response parameter estimation proceeds by comparison of the latter with the recursively estimated cortisol secretion rate \(\hat{Z}_C(t)\) \((i = 1, \ldots, n)\), assumed to be \(\hat{Z}_C(t)\) \(+\) error. Random effects (\(A\) values) in efficacy are included to accommodate pulse-by-pulse variation.

To allow for desensitization of a cortisol response, an allowance for change in the response mechanism, specifically, a possible midpulse shift in the dose response, is included (Fig. 3, middle). That is, for an amount of time \(M_{AonC}\) (a parameter to be estimated) following the onset of a cortisol pulse, one dose-response curve is followed; then 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)

\[
\hat{Z}_C(t) = \begin{cases} 
\eta_0 + \frac{\eta_3 + A_{AonC}^{(k)}}{1 + \exp\left(-\left[\eta_1^{UP} + \eta_2 \times F_A(t)\right]\right)} & \text{if } T_C^{(k)} \leq t < T_C^{(k+1)} \\
\eta_0 + \frac{\eta_3 + A_{AonC}^{(k)}}{1 + \exp\left(-\left[\eta_1^{DOWN} + \eta_2 \times F_A(t)\right]\right)} & \text{if } T_C^{(k)} \leq t < T_C^{(k+1)} \end{cases} + e_i \quad i = 1, \ldots, n
\]

Model 2: dose-response slope (adrenal sensitivity)

\[
\hat{Z}_C(t) = \begin{cases} 
\eta_0 + \frac{\eta_3 + A_{AonC}^{(k)}}{1 + \exp\left(-\left[\eta_1^{UP} + \eta_2 \times F_A(t)\right]\right)} & \text{if } T_C^{(k)} \leq t < T_C^{(k+1)} \\
\eta_0 + \frac{\eta_3 + A_{AonC}^{(k)}}{1 + \exp\left(-\left[\eta_1^{DOWN} + \eta_2 \times F_A(t)\right]\right)} & \text{if } T_C^{(k)} \leq t < T_C^{(k+1)} \end{cases} + e_i \quad i = 1, \ldots, n
\]

Model 3: asymptotic maximum (ACTH efficacy)

\[
\hat{Z}_C(t) = \begin{cases} 
\eta_0 + \frac{\eta_3 + A_{AonC}^{(k)}}{1 + \exp\left(-\left[\eta_1^{UP} + \eta_2 \times F_A(t)\right]\right)} & \text{if } T_C^{(k)} \leq t < T_C^{(k+1)} \\
\eta_0 + \frac{\eta_3 + A_{AonC}^{(k)}}{1 + \exp\left(-\left[\eta_1^{DOWN} + \eta_2 \times F_A(t)\right]\right)} & \text{if } T_C^{(k)} \leq t < T_C^{(k+1)} \end{cases} + e_i \quad i = 1, \ldots, n
\]

In Fig. 3, applications of models 1–3 are shown for the subject used for Fig. 2.

Maximum-likelihood estimation of the dose-response parameters is performed. Figure 3 (middle) displays results for the present subject. The solid line (logistic curve) denotes the estimated initial mean dose response, and the dashed line denotes the ending mean dose response (after time \(M_{AonC}\) from the cortisol pulse onset) feedforward function. Light dashed 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.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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