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Overnight ACTH-Cortisol Dose-Responsiveness: comparison with 24-hr data, metyrapone administration, and insulin-tolerance test in healthy adults

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Abstract

Objective—To estimate the dose-dependence of endogenous ACTH's stimulation of adrenal cortisol secretion overnight.

Design—Ten-minute sampling for ACTH and cortisol over 8 and 24 hr (N=17), after metyrapone administration (N=6), during an insulin tolerance test (N=7).

Subjects—Healthy adults.

Measurements—ACTH dose-responsive estimates.

Results—Twenty-four hr ACTH-cortisol concentration pairs yielded an estimated EC₅₀ (one-half maximally stimulatory ACTH concentration) of 5.1 (2.2–9.5) pmol/L [median (range)]. This did not differ from EC₅₀'s based upon 8 or 6-hr data [5.9 (3.5–11) and 7.5 (3.7–41) pmol/L] in the same individuals. ACTH efficacy (maximally stimutable cortisol secretion rate) was 8.4 (3.1–20), 11 (5.9–24) and 15 (5.9–22) nmol/L/min, when calculated over 24, 8 and 6 hr, respectively (P=NS). Adrenal sensitivity (slope term) was also consistent across sampling durations, viz., 14 (1.3–95), 18 (1.3–64) and 20 (1.3–64) slope units. Compared with placebo, metyrapone reduced ACTH efficacy from 11 (6.2–62) to 2.8 (1.5–4.5) nmol/L/min for cortisol (N=9, P<0.001), while increasing ACTH efficacy for 11-desoxycortisol from 2.3 (0.9–2.9) to 99 (70–218) nmol/L/min (N=6, P<0.01), thus affirming face validity. Combined ACTH and cortisol responses to hypoglycemia allowed an estimate of ACTH efficacy of 28 (22–81) nmol/L/min, compared with the control value of 8.7 (5.6–26), suggesting enhanced adrenal responsiveness.

Conclusions—The results suggest that endogenous ACTH-adrenal drive can be approximated from overnight 8-hr sampling of paired ACTH and cortisol concentrations. This strategy may have merit in clinical research in childhood, pregnancy, anxiety states, and frail elderly individuals, when ACTH injections are not desired.

Keywords

corticotrophic; feedback; human; adrenal

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Conflict of Interest Statement

There is no conflict of interest that could be perceived as prejudicing the impartiality of research.

Introduction

Dynamic mechanisms that regulate time-varying hormone concentrations include feedback (inhibition) and feedforward (stimulation) ¹. Feedback and feedforward are mediated in turn via implicit dose-response functions ^{2,3}. In endocrine systems, disruption of dose-dependent regulatory interactions results in pathophysiology and illness ⁴. For example, Cushing's disease is marked by impaired cortisol-dependent feedback restraint of ACTH secretion by tumoral corticotrope cells ⁵⁻⁷. Despite the significance of dynamic control, quantification of endogenous dose-response properties has been difficult clinically.

In principle, paired hormone agonist-response time series contain implicit information about endogenous feedforward (and feedback) dose-dependencies ⁴. Recent investigations of ACTH and cortisol concentration-time series have presented the concept that dose-responsiveness should be inherent in the pairwise dynamics ⁸⁻¹². In general, earlier authors have illustrated (but not calculated) the inferred relationship between peak ACTH and peak cortisol concentrations, thereby allowing indirect visual estimates of the EC₅₀ (effective ACTH concentration driving one-half maximal cortisol peaks). Visual inspection has limitations in the reproducibility and precision of parameter estimation. Moreover, in more precise physiological terms, time-varying ACTH concentrations (rather than peak values) drive adrenal cortisol secretion *per se* (rather than peak cortisol concentrations). The availability of an analytical tool to make such calculations has permitted dose-response estimation using paired 24-hr data for ACTH-cortisol and LH-testosterone ¹³. The strategy proposed here requires deconvolving the effector-concentration (*e.g.* ACTH) and secretory-response (cortisol) hormone concentration profiles to obtain time-varying ACTH concentrations and matching cortisol secretion rates, both corrected for hormone-specific elimination ^{4,14}. The next step is to relate the calculated input (ACTH) concentration pulses to output (cortisol) secretion rates via a nonlinear dose-function. Thereby, one obtains objective estimates of effector efficacy (maximal stimulatory effect), EC₅₀ (one-half maximally effective ACTH concentration) and response (adrenal) sensitivity (a slope term). The need to collect 24-hr paired ACTH-cortisol or LH-testosterone time series makes the original procedure costly and time consuming. Accordingly, the present studies compare overnight ACTH-cortisol dose-response estimates with those of 24-hr data; validate the methodology by metyrapone (steroidogenic inhibitor) administration; and contrast time-of-day controls with an insulin-tolerance test (ITT).

Methodology

Subjects

Seventeen paired 24-hr profiles were obtained earlier by sampling blood every 10 min beginning at midnight in healthy adults prescreened for any endocrine disorder, depression history, weight loss, recent stress, medication use or glucocorticoid exposure ¹⁵. Nine of these subjects also received multidose overnight metyrapone (2 g orally every 2 hr beginning at midnight), when metyrapone was clinically available.

In six other men, placebo or single-dose metyrapone (3.0 g) was administered orally at 0000 hr, given limited availability of metyrapone, as described ¹⁶. Plasma ACTH, beta-endorphin, cortisol and 11-desoxycortisol were assayed in 10-min samples from midnight onward for 8 hr. Seven other healthy men were subjected to an insulin tolerance test (ITT). The data comprised 10-min ACTH and cortisol measurements over 4 hr starting at 0800 hr ¹⁷.

None of these data has ever been analyzed in the present fashion. All sampling was performed after approval by the local Ethics Committee.

Analysis

Analyses involved paired ACTH-cortisol concentration time series from 30 different subjects. Construction of dose-response estimates proceeded via three steps: (1) automated deconvolution of ACTH-concentration time series as described recently, so as to reconstruct discretized reconvolution (fitted) ACTH-concentration time curves; (2) automated deconvolution of the matching cortisol concentration time series to obtain sample (every 10 min) cortisol secretion rates⁴; and (3) automated dose-response estimation of the nonlinear 4-parameter logistic function dose-response relating pulsatile ACTH concentrations to cortisol secretion rates¹³. The four dose-response parameters were ACTH efficacy, EC₅₀, adrenal sensitivity (slope of the dose-response function) and basal (underlying nonpulsatile cortisol secretion rate)³. For improved model fit, a 2-sensitivity downregulation construct was employed that includes a time delay and both a high and a low sensitivity estimate¹³. The high sensitivity estimate is used here.

Dose-response reconstruction was applied analogously to 6- and 8-hr subseries of the full 24-hr data in the 17 control subjects; viz., midnight to 0600 hr (37 paired samples), and midnight to 0800 hr (49 paired samples). Secondary endpoints were dose-response estimates made from overnight single-dose metyrapone (N=6), multidose metyrapone (N=9), 4-hr (N=7) ITT and 4-hr normal (0800-1200 hr) [N=7] data.

Assays

The same assays were used in all 30 subjects studied¹⁵⁻¹⁷. Plasma ACTH was measured using a two-site sandwich assay designed to detect intact ACTH molecules. The immunoradiometric assay consisted of a soluble ¹²⁵I-labeled (indicator) monoclonal antibody directed to the N-terminus of ACTH as well as a second polyclonal ACTH antibody directed to the C-terminus. The second antibody was covalently conjugated to biotin to react with avidin-coated plastic beads. All incubation reagents including antibodies, human ACTH standard, and avidin-coated beads were from Nichols Institute (Allegro IRMA, San Juan Capistrano, CA). Each sample was assayed in duplicate, and all samples from any one subject were assayed in the same run. Sensitivity of the IRMA was 1.0 pg/mL or 0.22 pmol/L, and intraassay precision was 3.2–5.8% (range of median intrasample coefficients of variation in all individuals). Cross-reactivity with β -endorphin, TSH, LH, FSH, GH or prolactin was less than 0.1%.

Cortisol was assayed using antibody-coated tubes and reagents obtained from Clinical Assays (Dade, Baxter-Travenol Diagnostics, Cambridge, MA). Each sample was assayed in duplicate, and all samples from any one subject were assayed together. Sensitivity was 0.5 μ g/dL (14 nmol/L), and intra-assay precision 2.5–4.7% (range of median values for all individuals). Interassay coefficients of variation were 3.4 and 5.6%.

Statistics

In the 17 adults, dose-response parameters were evaluated by 1-way ANOVA after natural-logarithmic transformation. The 3 factors in the ANOVA model were 6-, 8- and 24-hr parameter estimates. The parameters are efficacy, EC₅₀, sensitivity and basal. $P < 0.05$ was construed as significant. The signed-ranks (paired) nonparametric test was used to compare placebo/metyrapone effects or ACTH/ β -endorphin responses and the unpaired rank-sum test to compare ITT and 4-hr control data¹⁸. Data are presented as the median (range) in the tables, and as vertical dot plots or the geometric mean $\pm$ SEM in the figures.

Results

Figure 1A illustrates an 8-hr overnight (midnight to 0800 hr) cortisol secretion profile calculated by deconvolution [*top*], corresponding measured and time-shifted reconvolved (fitted) ACTH concentrations [*middle*] and unit-normalized deconvolved cortisol secretion and reconvolved ACTH concentration plots [*bottom*]. The dose-response model used the last pair of profiles for parameter estimation. Subjects were not significantly stressed in these paradigms, in that serum cortisol concentrations between 0200 and 0400 hr fell to 56–130 nmol/L. Figure 1B illustrates the 4-parameter (nondownregulated) and sensitivity downregulated dose-response fits in this subject. The latter model greatly enhanced goodness-of-fit ($P < 0.001$) in the group. Table 1 summarizes median (range) parameter values for all 17 control individuals, who had been sampled for 24 hr. Outcomes were compared using all 24 hr or only 6 hr or 8 hr overnight. ANOVA was employed to compare ln-transformed dose-response parameter estimates obtained from the 3 segments (6, 8 and 24-hr) of the paired ACTH-cortisol profiles. Basal cortisol secretion, ACTH EC₅₀, adrenal sensitivity and ACTH efficacy did not differ among the sampling regimens. In fact, the 8-hr overnight segment gave results similar to those obtained from the full 24-hr paired ACTH/cortisol profiles. In particular, sensitivity estimates correlated strongly ($R = 0.827$, $P < 0.001$) between 8-hr and 24-hr estimates. Goodness of fit defined by model SD (a measure of residual model error) also did not differ for 6, 8 and 24-hr data. Thus, except for the 4-hr ITT data, further analyses are presented for the sensitivity-downregulation model.

Single-dose overnight metyrapone compared with placebo administration in 6 adults reduced calculated ACTH efficacy for cortisol secretion from 21 (12–101) to 6.1 (2.8–48) nmol/L/min ($P = 0.002$), and concurrently increased ACTH efficacy for 11-desoxycortisol secretion from 2.3 (0.90–2.9) to 99 (70–218) nmol/L/min ($P = 0.003$). For comparison with these median (range) values, the geometric means $\pm$ SEM are given in Figure 2. In the same subjects, beta-endorphin was also assayed on the placebo day. Statistical analysis showed that adrenal sensitivity was 3-fold lower ($P = 0.028$) and EC₅₀ 2.7-fold higher ($P = 0.043$) for beta-endorphin than for ACTH. Higher EC₅₀ denotes lower potency. Nonstimulated (nonpulsatile) basal cortisol secretion estimates did not differ, serving thus as a negative control: Figure 3. These data demonstrate agonist (ACTH vs beta-endorphin) specificity of dose-response estimates.

Nine healthy individuals received repeated overnight doses of metyrapone and placebo on separate days. Figure 4 shows that the metyrapone compared with the placebo schedule by paired comparison: (i) decreased ACTH efficacy by 4-fold (to 25% of control values, $P < 0.001$), (ii) increased ACTH EC₅₀ by 2.7-fold ($P = 0.003$), (iii) diminished adrenal sensitivity by 8-fold ($P < 0.001$) and (iv) reduced basal cortisol secretion by 20-fold ($P < 0.001$). These data further support model validity.

In fourteen other normal adults, dose-response parameters for ACTH-cortisol drive were assessed during 4-hr ITT ($N = 7$) and control ($N = 7$) sampling every 10 min fasting (0800–1200 hr). The sensitivity dose-response downregulation model was significant in all 7 subjects compared with the no-downregulation model (overall $P < 0.001$). Data from the ITT group and 7 controls studied over the same 4-hr window are given in Table 2. ACTH efficacy was 3.2-fold higher [$P < 0.01$] (and EC₅₀ and sensitivity similar) during hypoglycemia compared with fasting control subjects analyzed identically.

Discussion

The present investigations support the utility of dose-response estimation using overnight/early morning 8-hr (paired) ACTH-cortisol concentration time series. Several findings

affirm this idea. First, ACTH EC₅₀ values so estimated (median 5.9 [3.5-11] pmol/L) are quite consistent with 24-hr data (Table 1). Second, EC₅₀ estimates are similar to visually interpolated values obtained by other investigators in independent subject groups, *viz.*, 5.6–13 pmol/L for peak ACTH/peak cortisol data^{9–12;19;20}, albeit higher than that determined in an *in vitro* guinea-pig bioassay, average 3.2 pmol/L²¹. Third, when metyrapone was used to inhibit the conversion of 11-desoxycortisol to cortisol, ACTH efficacy fell by 4-fold (*viz.*, by 75%). Concomitantly, calculated ACTH potency, adrenal sensitivity and basal cortisol secretion fell by 2.7 to 20-fold (medians). Fourth, estimated beta-endorphin potency was 3-fold (placebo) and 8-fold (metyrapone) lower than that of ACTH in the same subjects. Fifth, the regression coefficient for adrenal-sensitivity estimates using 24-hr and 8-hr data had a slope of 1.0 ± 0.18 ($P < 0.001$, $R = 0.827$). And, sixth, during insulin-induced hypoglycemia to stimulate ACTH/cortisol secretion, ACTH efficacy was higher than for fasting control data. Although interindividual variations in the dynamic ACTH-cortisol axis are large^{22–24} as recognized here also, the collective results suggest that overnight ACTH-cortisol concentration time-series pairs allow clinical investigational estimation of endogenous ACTH's dose-dependent effects. This new approach is viewed as complementary to other clinical-research tools for evaluating ACTH action. An advantage would be the absence of ACTH injection, and a disadvantage the need for paired ACTH/cortisol measurements.

Insulin-tolerance testing evoked marked output of ACTH and cortisol. Dose-response analysis over the limited 4-hr interval yielded a 3.2-fold higher estimate of ACTH efficacy during hypoglycemia than in fasting control subjects. An increase in maximal adrenal responsiveness during hypoglycemia could be due to stimulation of splanchnic autonomic neural inputs to the adrenal gland⁸. Indeed, neurogenic signals to the adrenal gland augment ACTH action in other species²⁵. This point will require further studies.

Caveats include the needs to corroborate the present inferences in larger cohorts (here $N=30$); estimate ACTH potency and efficacy as well as adrenal sensitivity during other types of physical, metabolic and psychological stressors; obtain criterion-based dose-response data using exogenous ACTH pulses that mimic endogenous ultradian and circadian ACTH release patterns; and evaluate age and gender effects. In the last context, a recent study using 24-hr data suggested that ACTH efficacy declines with age in men and rises with age in women²⁶. Moreover, ACTH action may depend upon body composition, stress, sex steroids and other clinical factors. The present approach provides one means to quantify the effect(s) of such factors. In any case, clinical studies should employ suitable control group(s) matched otherwise for age, gender, obesity, sex-hormone milieu and neuropsychiatric background^{26–30}. Confounding by differing hormone kinetics is reduced by the fact that the deconvolution step in the dose-response methodology adjusts for interindividual differences in hormone disappearance⁴. Given that an overnight sampling schedule can capture large physiologic variations in pituitary-adrenal secretion during the later hr of sleep and upon awakening, the present concepts may be useful in investigating endogenous ACTH-cortisol coupling in pregnancy, childhood, frailty and other clinical pathophysiologies. In noninvestigational contexts that permit ACTH or insulin injection, a standard clinical method should be used^{22–24;31–33}.

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Overnight ACTH and Cortisol Secretion Estimates

Figure 1.

Panel A. Illustrative 8-hr profiles of deconvolved 10-min cortisol secretion rates (top), measured ACTH concentrations and time-shifted reconvolved ACTH concentrations (middle) and overlaid reconvolved ACTH concentrations with deconvolved cortisol secretion rates (bottom). **Panel B.** Fitted (interrupted) and deconvolved (solid) cortisol secretion rates (top). Dose-response functions were estimated from the paired ACTH concentrations and cortisol secretion rates (N=49 paired 10-min data) using a nondownregulation (left bottom) and sensitivity-downregulation (right bottom) model [Methods]. Continuous curves signify onset of pulsatile ACTH action; interrupted curves

define down-regulated responses. The model SD values are given along with the P value for improved fit by the downregulation model.

Efficacy Validation via 11-beta Hydroxylase Inhibition

Figure 2.

Comparison between ACTH efficacy estimate after placebo (control) and metyrapone (steroidogenic inhibitor) administration. Both cortisol (*left*) and 11-desoxycortisol (*right*) were measured. Efficacy denotes the maximal extrapolated corticosteroid secretion rate. P values are paired estimates (N=6 individuals).

Specificity of ACTH-Cortisol Dose Response

Figure 3. Specificity of ACTH *vis-à-vis* beta-endorphin drive of pulsatile adrenal cortisol secretion. Sensitivity (slope), EC₅₀ and basal (nonpulsatile) secretion values are shown along with paired statistics (N=6 subjects). Higher EC₅₀ denotes lower potency.

Overnight Metyrapone Validation: ACTH-Cortisol Drive

No downregulation model (○ control ● metyrapone)

Figure 4.

Comparisons of ACTH-cortisol dose response estimates in 9 adults given placebo (P) and metyrapone (M) overnight. Pairwise contrasts are noted by P values (N=9 volunteers).

Table 1

Lack of impact of sampling duration on ACTH-cortisol dose-response estimates Duration of Sampling

Parameters	24 hr	8hr	6hr
Basal (nmol/L/min)	0.17 (0.028–2.2)	0.14 (0.028–0.90)	0.34 (0.028–2.9)
EC ₅₀ (pmol/L)	5.1 (2.2–9.5)	5.9 (3.5–11)	7.5 (3.7–41)
Sensitivity (slope units)	14 (1.3–95)	18 (1.3–64)	20 (1.3–64)
Efficacy (nmol/L/min)	8.4 (3.1–20)	11 (5.9–24)	15 (5.9–22)
Model SD ^I (error term)	5.6 (3.9–9.0)	6.7 (2.0–11)	6.7 (3.1–18)

Data are median (range) in 17 subjects.

^I
nmol/L/min

Table 2

ACTH-cortisol dose-responsiveness estimated during ITT vs fasting

Parameters	Clinical Condition	
	ITT	Fasting
Basal ¹	0.11 (0.03–2.1)	1.1 (0.25–1.6)
EC ₅₀ ²	7.7 (0.26–58)	2.9 (0.05–6.2)
Sensitivity ³	7.4 (1.4–241)	56 (13–330)
Efficacy ¹	28 (22–81) ⁴	8.7 (5.6–26)
Model SD ¹	4.2 (3.1–6.7)	2.8 (2.6–5.6)

Four-hr sampling was performed (0800–1200 hr) in both ITT (N=7) and fasting control (N=7) subjects.

Data are median (range) for ACTH-cortisol dose-response parameters in the sensitivity-down-regulation model.

¹ nmol/L/min

² pmol/L

³ slope units

⁴ P<0.01 vs fasting