Sex defines the age dependence of endogenous ACTH-cortisol dose responsiveness

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1Department of Statistics, University of Virginia, Charlottesville, Virginia; 2Department of Endocrinology and Metabolic Diseases; Leiden University Medical Center, The Netherlands; 3Pacific Behavioral Research Foundation, Carmel, California; 4Endocrine Service, Medical Section, Salem Veterans Affairs Medical Center, Salem, Virginia; and 5Endocrine Research Unit, Mayo Medical School, Mayo School of Graduate Medical Education, Center for Translational Science Activities, Mayo Clinic, Rochester, Minnesota

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Keenan DM, Roelfsema F, Carroll BJ, Iranmanesh A, Veldhuis JD. Sex define the age dependence of endogenous ACTH-cortisol dose responsiveness. Am J Physiol Regul Integr Comp Physiol 297: R515–R523, 2009. First published June 17, 2009; doi:10.1152/ajpregu.00200.2009.—Sex influences adrenal glucocorticoid responses to ACTH in experimental animals. Whether similar sex differences operate in humans is unknown. To test this notion, we estimated ACTH-cortisol dose-response properties analytically in 48 healthy adults (n = 22 women, n = 26 men); ages 18–77 yr, body mass index (BMI) 18–32 kg/m2; previously studied at two medical centers. Plasma ACTH and cortisol concentrations were measured every 10 min for 24 h. The 145 sample pairs were used in each subject to estimate ACTH-cortisol drive via a logistic function. Statistical analyses revealed that 24-h cortisol secretion (>82% pulsatile) fell in men (r = −0.38, P = 0.028) and rose in women (r = +0.37, P = 0.045) with age (P = 0.01 sex effect). The mechanisms involved decreased ACTH efficacy with age in men (r = −0.35, P = 0.04), and increased ACTH efficacy with age in women (r = +0.42, P = 0.025) [P = 0.009 sex effect]. ACTH potency diminished with higher BMI in men (r = +0.38, P = 0.029) and in the cohort as a whole (r = 0.34, P = 0.0085). These outcomes demonstrate that sex, age, and BMI modulate selective properties of endogenous ACTH-cortisol drive in humans, thereby indicating the need to control these three major variables in experimental comparisons.

Adrenal; aging; sex; men; women; secretion

In experimental animals, estrogens sensitize and androgens mute corticotropin-axis responses to stress (15, 21, 60, 75). Data in the human are indirect and controversial. For example, in one study in eight hypogonadal men, cortisol production rates were normal (77), whereas in another study, leuprolide-induced gonadopitvation unmasked greater ACTH and cortisol responses to corticotropin-releasing hormones (CRH) in young and middle-aged men than premenopausal women (59). Analyses of young hypogonadal adults further suggest that serotoninergic agonists and psychosocial stress stimulate greater corticotropin-adrenal responses in young men than young women (49, 61). On the other hand, cortisol concentrations are reportedly higher in women than men during early sleep, dexamethasone suppression, interleukin-6 infusion, growth hormone-releasing hormone (GHRH) injection, CRH stimula-

Overview

To date, no clinical studies have appraised the individual and interactive effects of sex and age on adrenal responses to endogenous pulses of ACTH. Indirect estimates of adrenal responsivity have used the ratio of plasma cortisol to ACTH concentrations, and thereby inferred elevated, equivalent, or reduced cortisol/ACTH ratios in older compared with young adults and/or in women compared with men (2, 8, 19, 26, 33, 43, 48). However, interpretation of cortisol/ACTH ratios assumes a simple linear relationship between ACTH and cortisol concentrations. This simplifying assumption is demonstrably incorrect, inasmuch as increasing ACTH concentrations (or doses) drive nonlinear (asymptotic, saturable) increases in cortisol concentrations in animals and humans (51, 52, 69).

A recent analytical method allows noninvasive estimation of physiological (nonlinear) dose-response functions from serially sampled hormone effector-response pairs, such as LH and testosterone, GnRH, and LH, or ACTH and cortisol without the injection of hormone agonists, antagonists, or labeled compounds (13, 34). This new tool is used to assess the unknown effects of sex and age on specific attributes of ACTH’s concentration-dependent drive of cortisol secretion in 48 healthy adults over 24 h.

METHODS

Overview

Paired 10-min plasma ACTH and cortisol concentration-time series collected over 24 h in 48 healthy adults from two centers were reanalyzed (11, 47). The present analysis does not overlap with earlier outcomes or methods in any manner. ACTH efficacy and potency were estimated analytically in each subject, then regressed on age in
women and men. The null hypothesis was that sex does not determine the effect of age on endogenous ACTH efficacy or potency.

**Human Subjects**

Healthy, unmedicated volunteers participated in the study (26 men and 22 women). Each subject provided written informed consent, and the protocol was approved by the Institutional Review Board. The age range was 18–77 yr. Participants maintained conventional work and sleeping patterns and reported no recent (within 10 days) transmeridian travel, weight change (>2 kg in 6 wk), shift work, intercurrent psychosocial stress, prescription medication use, substance abuse, neuropsychiatric illness, or acute or chronic systemic disease. A complete medical history, physical examination, and screening tests of hematological, renal, hepatic, metabolic, and endocrine function were normal. No subject had been exposed to glucocorticoids within the preceding 3 mo.

Volunteers were admitted to the Study Unit the evening before sampling for adaptation. Premenopausal women were studied in the follicular phase. No women were receiving estrogens, and no men were receiving androgens. Ambulation was permitted to the lavatory only. Vigorous exercise, daytime sleep, snacks, caffeinated beverages, and cigarette smoking were disallowed. Meals were provided at 0800, 1230, and 1730, and room lights were turned off between 2200 and 0600 depending upon individual sleeping habits. Blood samples (2.0 ml) were withdrawn at 10-min intervals for 24 h beginning either at midnight (17 subjects) or at 0900 (31 subjects). Blood was collected in prechilled siliconized tubes containing EDTA (ACTH) or heparin (morning, each blood sample was processed immediately). Cortisol was assayed by high-pressure liquid chromatography with ultraviolet detection (25). Sensitivity (25 nmol/l) solid-phase RIA (Sorin Biomedica, Milan, Italy). Intra-assay and interassay CVs were 5.1 and 6.4%, respectively. Cortisol was assayed by high-pressure liquid chromatography with ultraviolet detection (25).

**Laboratory Assays**

Plasma ACTH concentrations were quantitated in duplicate by high-sensitivity (3 ng/l) and high-specificity double-monoclonal immunoradiometric assay, using reagents from Nichols Diagnostics Institute (San Clemente, CA) between the years 1994 and 1997. Median within- and between-assay coefficients of variation of (CVs) were 5.3 and 6.5%, respectively. Cortisol was assayed by high-sensitivity (25 nmol/l) solid-phase RIA (Sorin Biomedica, Milan, Italy). Intra-assay and interassay CVs were 5.1 and 6.4%, respectively. No samples were undetectable in either assay. Current ACTH assays may give similar or considerably lower results that the erstwhile Nichols assay (57, 79).

**Analytical Formulation**

The analytical objective was to estimate properties of the in vivo dose-response relationship mediating pulsatile ACTH concentration-dependent drive of cortisol secretion over 24 h in individual subjects without administering an agonist, antagonist, or labeled marker (34, 35). The core model equations provide a representation of admixed basal and pulsatile secretion, cortisol subject-specific slow-phase half-lives with a fixed rapid-phase half-life of 2.4 min comprising 37% of the decay amplitude, allowable random effects on successive cortisol secretory-burst mass values, and experimental uncertainty due to sample withdrawal, processing, and assay (35, 36). Key features are highlighted below.

**Secretion and elimination functions.** Time-varying cortisol concentrations, \( X(t) \), can be described by the solution to a set of coupled differential equations incorporating basal (time-invariant) and pulsatile (burst-like) secretion and exponential elimination. Total secretion is given by the sum of basal and pulsatile: 

\[
Z(\cdot) = \beta_0 + P(\cdot),
\]

as follows:

\[
X(t) = \left[ a e^{-\alpha_1 t} + (1 - a) e^{-\alpha_2 t} \right] X(0) + \int_0^t \left[ a e^{-\alpha_1 (t-r)} + (1 - a) e^{-\alpha_2 (t-r)} \right] Z(r) dr
\]

\[
\approx \beta_0 \frac{a}{\alpha_2} \left( 1 - e^{-\alpha_2 t} \right) + \frac{1 - a}{\alpha_2} \left( 1 - e^{-\alpha_2 t} \right)
\]

\[
+ \int_0^t \left[ a e^{-\alpha_1 (t-r)} + (1 - a) e^{-\alpha_2 (t-r)} \right] P(r) dr
\]

“concentration due to basal secretion” + “concentration due to pulsatile secretion,” where \( a \) is the proportion of rapid to total elimination (0.37), \( \alpha_1 \) and \( \alpha_2 \) are the respective rate constants of the rapid (0.298) and slow (estimated) elimination phases, \( X(0) \) is the starting hormone concentration, \( \beta_0 \) is the basal secretion rate, \( t \) time and \( P(t)dr \) are the instantaneous pulsatile secretion rate over the infinitesimal time interval \( r, r + dr \) (35, 36).

The function defining pulsatile cortisol secretion was given by

\[
P(t) = \sum_{i \in S} M_i \psi(r - T_i), r \geq 0
\]

where \( \psi(s) \) denotes the wave function (burst shape), a three-parameter generalized gamma probability distribution normalized to integrate to unity; \( M_i \) denotes the (deterministic plus random) mass of cortisol released per unit distribution volume in the \( j^{th} \) burst; \( \eta_i \) is the fixed basal cortisol synthesis rate in the adrenal gland; \( \gamma_i \) is the rate of additional cortisol accumulation over the time interval, \( T_i - T_{i-1} \); and, \( A_i \) are random effects on the mass of the \( j^{th} \) cortisol burst. Asymmetric and symmetric (e.g., Gaussian) secretion events are well approximated by the three-parameter gamma density [above psi function]. Such flexibility is important, inasmuch as discrete pulses of ACTH evoke rapid onset and prolonged cortisol secretion in vivo in the sheep, rat, and dog and in vitro during perfusion of adrenal cells (5, 25).

**Dose-response estimation.** A statistical basis exists for analytical estimation of four-parameter logistic functions relating time-varying agonist (ACTH) concentrations to target-gland (adrenal cortisol) secretion rates (34–36). One parameter is simply basal secretion. The two main parameters are efficacy and potency, as defined in Primary model parameters. Experimental validation was accomplished by frequent (5 min) and extended (4–12 h) direct sampling of hypothalamo-pituitary portal and jugular venous blood in the unrestrained, conscious unmedicated sheep and horse, and repetitive sampling (every 20 min for 17 h) of LH and testosterone concentrations in the human spermatic vein (34, 35). Statistical verification was by formal mathematical proof of unbiased asymptotic properties of maximum likelihood-based estimates (MLE) of all parameters simultaneously (13). The motivation for and detailed methodological steps used in estimating a four-parameter logistic dose-response function are given for the reader by Keenan and Veldhuis (37).
RESULTS

General subject characteristics are given in Table 1. Figure 1 depicts measured (dark line) and model-estimated (lighter line) plasma cortisol concentrations sampled every 10 min for 24 h in a 24- and a 76 yr-old woman (A, top) and in a 26- and a 58-yr-old man (B, top). Matching cortisol secretion rate estimates (upper middle) and measured (untransformed) ACTH concentrations (lower middle) are given. The analytical model relates measured ACTH concentrations (pmol/l) via an estimated four-parameter dose-response function to cortisol secretion rates (nmol·l⁻¹·min⁻¹) calculated simultaneously (bottom). In each subject, multiple dose-response curves are plotted, each with a different efficacy. The curves reflect random effects on the mean efficacy (solid line) due to small response variations among different ACTH/cortisol pulse pairs in that person (interrupted lines). To be representative, profiles include data from midnight to midnight (A) and from 0900 to 0900 (B). Axes are scaled to highlight differences in the dynamics.

Linear regression analysis was used to evaluate whether sex influences how age alters ACTH-cortisol dose-responsiveness for 1) all 48 subjects, 2) men only (n = 26), and 3) women only (n = 22): Fig. 2. The two primary dose-response parameters were ACTH ED₅₀ (potency) and ACTH efficacy (asymptotically projected maximal cortisol-secretory response). ACTH efficacy correlated with age negatively in men (P = 0.025) and positively in women (P = 0.040). The slopes in men and women differed significantly (P = 0.009 by two-tailed Student’s t-test). Thus, sex affects the age dependence of ACTH efficacy in this cohort. This outcome was specific, since regressions of ACTH ED₅₀, and secondarily adrenal sensitivity and basal cortisol secretion on age, yielded no age or sex dependencies (Table 2). The opposite effects of age on ACTH efficacy in men and women were reflected in analogous differences in both total (pulsatile plus basal) daily cortisol secretion (sex effect P = 0.01) and the mass of cortisol secreted per burst (sex effect: P = 0.03). In particular, age diminished ACTH drive in men (P = 0.028 for total cortisol secretion, P = 0.045 for cortisol mass per burst), whereas age augmented total cortisol secretion in women (P = 0.045). Neither age nor sex affected percentage pulsatile cortisol secretion (mean 84 ± 1.3%; n = 48), cortisol half-life (53 ± 2.5 min) or secretory-burst frequency (21 ± 1 pulses/day).

A secondary postulate was that BMI influences ACTH-cortisol dose-response coupling. As depicted in Fig. 3, BMI was a significantly positive correlate of ACTH ED₅₀ (potency) in the cohort as a whole (r = 0.34, P = 0.0085, n = 48). The same was true in men (r = 0.38, P = 0.029), but not in women (P = 0.15). The male/female slope difference for the BMI effect on ACTH potency was not significant. Sensitivity to ACTH, ACTH efficacy, basal cortisol secretion, cortisol half-life, total cortisol secretion, cortisol secretory-burst frequency, and mass were unrelated to BMI over the range 18–32 kg/m².

DISCUSSION

The present paradigm complements a concept of dose-dependent ACTH-cortisol drive (here designated as feedforward) developed in 1983 by Keller-Wood et al. (39) and based upon 40-min infusions of dose-varying α-1,24-ACTH in dogs. The current paradigm differs by comprising paired 24-h endogenous ACTH and cortisol times series based upon 10-min sampling (145 samples of both hormones), and a noninvasive analytical model to estimate feedforward (stimulatory) properties of endogenous ACTH-concentration pulses on cortisol secretion in 48 healthy adults ages 18–77 yr with BMI’s of 18–32 kg/m². Salient outcomes are that sex determined the effects of age on I) total (pulsatile plus basal) daily cortisol secretion, 2) the stimulatory efficacy of ACTH, and 3) the mean amount (mass) of cortisol secreted per burst. Each of the three measures increased with age in women, but decreased with age in men. Figure 4 illustrates this by showing ACTH-cortisol dose-response functions by age tertile and sex. The opposite effects of age on ACTH efficacy in men and women are not readily explicable by the known trend of cortisol-binding globulin (CBG) concentrations to decline with age in both sexes and to decrease in a low estrogenic milieu in women (41, 55, 66). The sex effects were selective, because sex did not affect ACTH potency, the slow-phase half-life of cortisol, basal (nonpulsatile) cortisol secretion, secretory-burst frequency, or estimated adrenal sensitivity to ACTH (Table 3).

The strong effects of sex on age’s determination of total cortisol secretion were reflected in corresponding differences in the amount of cortisol secreted per burst. ACTH efficacy in the present construction denotes analytically extrapolated asymptotically maximal pulsatile cortisol secretion. In the cohort evaluated here, cortisol secretory-burst size (nanomoles of cortisol released per ACTH-associated secretory burst per liter distribution volume) increased in parallel with ACTH efficacy. This finding is not dissimilar to the net experimental outcome in dogs, wherein higher ACTH doses increase total cortisol secretion by initially increasing the height and subsequently also prolonging the duration of the cortisol secretory response, thereby together increasing total cortisol secretion (39). In the
present model, although the duration of individual cortisol pulses was not estimable, the total amount (mass) of cortisol secreted per burst is the integral of the secretion pulse (similar to a height-width product) (37). The estimated mass per pulse is multiplied by the number of ACTH pulses to obtain total pulsatile cortisol secretion. ACTH pulse frequency did not change with age and did not differ by sex. Thus, age and sex primarily modulate the amount of cortisol secreted in bursts.

Fig. 1. Illustrative ACTH-cortisol concentration-secretion profiles and dose-response estimates in a young and older woman (A) and a young and older man (B). Top row: measured plasma cortisol concentrations sampled every 10 min over 24 h (dark lines) and model-estimated values (light lines) (multiply nmol/l cortisol by 0.0363 to obtain μg/dl). Upper-middle row: cortisol secretion rates. Lower-middle row: ACTH concentrations (multiply pmol/l by 4.5 to convert to ng/l or pg/ml). Bottom row: ACTH concentration dose-cortisol secretory-response curves reconstructed for the set of ACTH pulses identified in each subject (interrupted lines). The solid dark line is the subject’s mean dose-response estimate over 24 h. Note different scales to visualize the dynamics.
New analytical models will be needed to discern the extent to which individual cortisol secretory-burst shape (waveform) and size (amplitude) are determined by ACTH concentrations. Since our data were not obtained after imposition of stressors, we cannot readily predict how ACTH-cortisol relationships would change with age and sex in the presence of illness, disease, or experimental stress.

The basis for higher estimated ACTH efficacy in young men and aging women is not yet known. This cannot be explained by CBG differences, since CBG is actually greater in young women than young men, and declines with age in both sexes (41, 55, 66). Estrogen potentiates and testosterone represses adrenal steroidogenic responses to exogenous ACTH in animal models (15, 60). The same sex-steroid effects on adrenal responsivity to ACTH have not been demonstrated directly in primates. However, the observation that sex differences in cortisol availability exist even in prepubertal children and hypogonadal adults (28, 59) would suggest that either sex steroids do not mediate sex differences in adrenal responsiveness or that their effects are exerted early in life and sustained. Further studies will be required to address these considerations.

The rise in calculated ACTH efficacy with age in women is consistent with measurements of cortisol concentrations and production rates in several clinical studies (27, 43, 48, 48, 54). Conversely, the fall in ACTH efficacy with age in men is consistent with declining cortisol levels inferred in other investigations (2, 16, 24, 40, 43, 49, 55, 61, 63, 64, 78). Our finding of opposing effects of age on ACTH-cortisol efficacy in men and women explain the absence of an overall effect of age when data from both sexes are considered together. The present outcomes supported by some (12, 32, 46, 53, 62, 81), but not other (18, 48, 55, 70), indirect analyses. Contradictory earlier data may reflect in part the influence of stressor type on sex differences (71); small numbers of subjects studied (40, 59, 77, 78); morning vs. late-day sampling (72); and possible effects of ethnicity (14, 80).

BMI was used as a surrogate of relative adiposity. Previous reports suggest variously that BMI does not predict (53),

Table 2. **Summary of Regression Statistics on Age**

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 26)</th>
<th></th>
<th>Women (n = 22)</th>
<th></th>
<th>Gender Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Cortisol Secretion</td>
<td>Cortisol Mass per Pulse</td>
<td>ACTH Efficacy</td>
<td>Total Cortisol Secretion</td>
<td>Cortisol Mass per Pulse</td>
</tr>
<tr>
<td>Correlation</td>
<td>−0.38</td>
<td>−0.34</td>
<td>−0.35</td>
<td>0.37</td>
<td>0.29</td>
</tr>
<tr>
<td>Slope</td>
<td>−66.7</td>
<td>−3.0</td>
<td>−0.24</td>
<td>45.3</td>
<td>1.7</td>
</tr>
<tr>
<td>SE</td>
<td>33.1</td>
<td>1.7</td>
<td>0.13</td>
<td>25.5</td>
<td>1.2</td>
</tr>
<tr>
<td>t</td>
<td>−2.02</td>
<td>−1.76</td>
<td>−1.82</td>
<td>1.78</td>
<td>1.37</td>
</tr>
<tr>
<td>df</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>P value</td>
<td>0.028</td>
<td>0.045</td>
<td>0.04</td>
<td>0.045</td>
<td>0.09</td>
</tr>
</tbody>
</table>
cortisol distribution volumes of 8.4 and 8.0 l/m² and body surface areas of 1.73 and 1.60 m², respectively (9, 42)]. In contrast, analytically estimated ACTH efficacy (defined by asymptotically maximal ACTH-stimulated pulsatile cortisol secretion) would be 136 and 113 μmol/day in men and women, respectively. These estimates imply that, according to an asymptotic dose-response model, mean projected maximal endogenous cortisol secretion is about 8.5-fold the unstimulated cortisol secretion rate. Reported (free) cortisol responses to maximal stress (e.g., sepsis or near exsanguination) fall in this range, namely, 6–12-fold elevations (6, 7, 10, 31). Thus, the asymptotic logistic model applied here in uninfused humans can provide seemingly reasonable predictions.

In five available clinical papers that allowed post hoc estimation of ACTH potency, the mean was 8.2 (range 5.6–13) pmol/l (3, 4, 51, 52, 69). These indirectly inferred values are slightly higher than the present analytical estimate of 5.3 ± 1.9 (SD) and 4.2 ± 1.9 pmol/l, in men and women, respectively. ACTH potency in a guinea-pig bioassay is 3.2 pmol/l (44). Model-based estimates obtained in the 48 subjects studied here closely approximated mean (24-h) ACTH concentrations in the same individuals (3.7–4.7 pmol/l). Accordingly, we postulate that ACTH concentrations fluctuate around and near in vivo ACTH potency, as estimated by the noninvasive methodology. An analogous inference has been made for luteinizing hormone-testosterone coupling in vivo (34, 38). In all, the data suggest utility of the present analytical method for investigating other agonist-driven physiological systems in vivo.

In conclusion, noninvasive analytical estimation of endogenous ACTH concentration-dependent drive of pulsatile cortisol secretion delineates sex-by-age interactions in determining ACTH efficacy, the mass of cortisol secreted per burst, and 24-h pulsatile (but not basal) cortisol secretion in healthy adults. The ensemble data introduce a potentially unifying hypothesis, in which age potentiates ACTH-adrenal coupling in women while attenuating the same in men.

**Perspectives and Significance**

Endocrine glands signal via putatively nonlinear dose-response functions, as predicted on theoretical grounds for ligand-receptor interactions with coupled second and third-messenger systems (17). The conventional experimental procedure for estimating such interactions is to infuse exogenous, or nullify endogenous, agonists, and measure concentration-dependent responses by the target organ. Usually the agonist is infused repeatedly on separate occasions to avoid confounding by possible response desensitization. A (logistic) dose-response function is then constructed from a collection of five or six dose-response points.

### Table 3. Summary of Estimated ACTH-Cortisol Dose-Response Parameters

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 26)</th>
<th>Women (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH: efficacy, nmol/l/min</td>
<td>6.7 (5.5)±0.77</td>
<td>6.3 (5.1)±0.72</td>
</tr>
<tr>
<td>ED₅₀ (potency), min</td>
<td>5.3 (5.1)±0.38</td>
<td>4.2 (4.0)±0.38</td>
</tr>
<tr>
<td>Cortisol: total secretion, nmol/l/day</td>
<td>1297 (1132)±119</td>
<td>1132 (1049)±102</td>
</tr>
<tr>
<td>Cortisol: basal secretion, nmol/l/day</td>
<td>243 (226)±33</td>
<td>207 (190)±19</td>
</tr>
<tr>
<td>Mass per pulse, nmol/l</td>
<td>63 (52)±6.1</td>
<td>55 (47)±4.7</td>
</tr>
<tr>
<td>Cortisol: slow half-life, nmol/l</td>
<td>57 (56)±3.1</td>
<td>45 (45)±2.9</td>
</tr>
</tbody>
</table>

Data are expressed as means (median) ± SE. MPP, mass per pulse.
more agonist doses and matching responses. The present strategy instead is to relate endogenously generated agonist pulses to corresponding endogenous (secretory) responses via an analytically estimated dose-response function. Two advantages of this new analytical approach are 1) the dose-response function so estimated in the uninfused host should capture the effects of truly physiological agonist signals, which are difficult to mimic precisely by exogenous infusions, and 2) endogenous (but not necessarily exogenous) agonist pulses occur at physiological time intervals, which should limit artefacts otherwise caused by the random relationship between exogenous pulses and the state of responsiveness or refractoriness of the target organ. An example of the former problem is infusing ACTH continuously to test adrenal responses, when, in fact, ACTH is secreted in distinct pulses possibly synchronized with adrenal innervation (22). An example of the latter is the infusion of growth hormone-releasing hormone without knowledge of adrenal innervation (22). An example of the latter is the infusion of growth hormone-releasing hormone without knowledge of adrenal innervation (22).

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REFERENCES

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