Secretory process regularity monitors neuroendocrine feedback and feedforward signaling strength in humans

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Veldhuis, J. D., M. Straume, A. Iranmanesh, T. Mulligan, C. Jaffe, A. Barkan, M. L. Johnson, and S. Pincus. Secrecy process regularity monitors neuroendocrine feedback and feedforward signaling strength in humans. Am J Physiol Regulatory Integrative Comp Physiol 280: R721–R729, 2001.—The present experiments examine the neuroregulatory hypothesis that the degree of sample-by-sample regularity of hormone output by an interlinked hypothalamopituitary target-organ system monitors the strength of feedback and/or feedforward signaling. To test this postulate and assess its generality, we implemented a total of nine thematically complementary perturbation experiments. In particular, we altered feedback or feedforward signaling selectively in two distinct neuroendocrine systems; namely, the growth hormone (GH) insulin-like growth factor type I (IGF-I) and the luteinizing hormone-testosterone axes. Four experimental paradigms comprised preferential reduction vs. enhancement of IGF-I or testosterone feedback signal strength; and, conversely, five others entailed selective attenuation vs. augmentation of GH-releasing hormone and gonadotropin-releasing hormone feedforward signal intensity. In these independent interventions, quantitation of subordinate (nonpulsatile) secretory pattern reproducibility via the approximate entropy statistic unmasked salient parameter output mirrors attenuated feedback and/or augmented feedforward coupling within an integrative system.

neuroregulation; growth hormone; luteinizing hormone; insulin-like growth factor type I; testosterone; thyrotropin; thyroxine; ACTH; cortisol; hormone

UNDER PHYSIOLOGICAL CONDITIONS, neuroendocrine axes are believed to operate as complex feedforward and feedback control systems (55, 66). For example, in the case of the growth hormone (GH) insulin-like growth factor type I (IGF-I) axis, hypothalamic peptidergic signals exert stimulatory or inhibitory effects on anterior pituitary somatotropes, and secreted GH and IGF-I impose time-delayed negative feedback on hypothalamopituitary feedforward drive (7, 11). Likewise, in relation to the male and female reproductive axes, the arcuate-nucleus neuropeptide gonadotropin-releasing hormone (GnRH) activates pituitary secretion of luteinizing hormone (LH), which stimulates gonadal sex-steroid hormone production. Androgens and estrogens, in turn, feed back negatively to restrain output of the GnRH-gonadotrope unit (8, 23, 54). Such dynamically coupled neuroendocrine systems presumptively achieve homeostasis via axis-specific, nonlinear, time-delayed, and dose-responsive feedback and feedforward linkages. An implicit corollary thesis is that such interactive properties dictate the unique time-dependent secretory patterns of each axis (22). According to this perspective, pathophysiologic disruption of hormone secretion could arise from defects within a control locus itself and/or by way of failure of internodal (pathway level) communication (40, 42, 62).

Assessing alterations in the behavior of integrated biological networks is critical, especially to an understanding of pathophysiology. Unfortunately, direct experimental assessment of complex systems is generally remarkably difficult, particularly in multinodal neuroendocrine axes (1, 2, 6, 9, 23, 33, 40–42, 45, 62). Full or multisite network data are rarely available. Moreover, even when several network components are measurable simultaneously, acquiring multiple concurrent data sets may be prohibitively expensive and/or invasive, thereby limiting analyses and/or disrupting normal system behavior. Yet quantitation of system-level change is crucial to elucidating the nature of potential network disruptions, whether due to alterations in

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feedback and/or feedforward signaling pathways (34, 35). Thus an important issue in integrative physiology is whether one can appraise system feedback changes indirectly and in a statistically valid manner simply by observing a single variable.

Thematically, the approximate entropy (ApEn) measure quantifies feedback changes within a closed but interactive system, as validated in several reductionist mathematical contexts (23, 33, 34, 39, 41). ApEn is a two-parameter family of regularity statistics, designed to contrast the degree of subordinate regularity in system output, as quantified by subpattern reproducibility in time series (METHODS). In various numerically coupled model forms, the quantifiable irregularity (and, thus ApEn) of signal output increases with increasing positive feedback and, conversely, decreases with increasing negative feedback (DISCUSSION). On the basis of this background, herein we examine whether quantitation of secretory pattern orderliness with ApEn can monitor feedback alterations in a variety of human neuroendocrinologic networks. In corollary, we explore how consistently ApEn-quantified changes in serial hormone regularity match theoretical predictions.

METHODS

Overview

The following published experiments were used to examine the utility of ApEn to discriminate differences in the orderliness of hormone secretory patterns induced by selected experimentally imposed variations in feedback and/or feedforward signaling: 1) exogenously imposed feedback enhancement, as achieved by constant peripheral intravenous infusion of IGF-I to suppress GH production (19) and of testosterone to repress pulsatile LH secretion (64); 2) muted endogenous negative feedback signaling, as accomplished in the GH axis by systemic IGF-I depletion (3) and in the GnRH-LH axis by inhibition of testosterone biosynthesis (64, 67); 2) augmented exogenous feedforward drive, as imposed by pulsatile fixed-dose and fixed-interval intravenous infusions of the hypothalamic releasing factors GH-releasing hormone (GHRH) or GnRH to augment GH and LH output (17, 30); and 4) attenuated endogenous hypothalamic feedforward input, as enforced by infusion of somatostatin or a selective peptidyl antagonist of the GnRH receptor in the GH axis and of a downregulating agonist of the GnRH receptor in the case of the LH axis (18).

Specific Clinical Protocols

IGF-I and testosterone feedback augmentation. Serum GH concentration time series were obtained by frequent (10 min) blood sampling for 24 h during paired and randomly ordered saline vs. recombinant human (rh) IGF-I infusions (10 μg·kg⁻¹·h⁻¹) in eight young men and seven young women, as published earlier (19). No ApEn data have been reported for these studies. Serum GH concentrations were quantitated in each sample by ultrasensitive chemiluminescence-based assay, which detected GH in all samples (15, 60).

Serum LH concentration profiles were obtained by sampling blood every 10 min during the second 24 h of the randomly ordered administration of placebo or 1,600 mg ketoconazole (KTCZ) given orally daily for 48 h in eight healthy young men to block androgen biosynthesis. In a subset of six individuals, a continuous intravenous infusion of saline or 8.0 mg testosterone was used to replete androgen during KTCZ ingestion (64, 67). LH was measured by two-site immunoradiometric assay (IRMA) (53).

IGF-I and testosterone feedback withdrawal. Plasma IGF-I concentrations were lowered experimentally in eight young midluteal-phase women by imposing a 2.5-day fast (3). Blood was sampled every 10 min during the last 24 h of the randomly ordered paired fed vs. fasting sessions. Serum GH concentrations were measured by chemiluminescence assay (above).

GHRH and GnRH feedforward augmentation. GHRH feedforward was accentuated in 19 men by infusing GHRH (0.33 μg·kg⁻¹·bolus) vs. saline intravenously every 90 min for 3 days. Blood was sampled at 10-min intervals for 24 h on the third day for later chemiluminescence-based assay of serum GH concentrations (17).

GnRH stimulation of LH secretion was augmented in five other young men by pulsatile infusion of this decapeptide (100 μg·bolus) vs. saline intravenously every 90 min for 14 days via a portable pump. LH was assayed by IRMA in sera collected every 10 min for 24 h on day 14 (30).

GHRH and GnRH feedforward inhibition. GHRH feedforward was antagonized by a bolus intravenous injection followed by continuous infusion of a specific antagonist of the GHRH receptor or saline for 8 h overnight in six young men (18). In other experiments, GHRH feedforward was opposed via a constant 3-h intravenous infusion of somatostatin-14 (30 μg·1.73 m⁻²·h⁻¹) in 10 postmenopausal women (5). Serum GH samples were collected every 10 min and later assayed by chemiluminescence (above).

GnRH feedforward was impeded in six young men by subcutaneous injection of an LH-downregulating dose of a long-acting GnRH agonist (leuprolide acetate 3.75 mg) 3–4 wk before blood sampling. Blood was withdrawn at 10-min intervals for 12 h (0800–2000). Serum LH concentration time series were compared with those obtained in 13 other age-matched, saline-treated men, who underwent an identical blood-sampling protocol. Serum LH concentrations were measured by IRMA (31, 53).

All samples contained uniformly detectable serum GH or LH concentrations in each of the above nine studies.

ApEn

ApEn is a translation- and scale-independent regularity measure that quantifies the serial regularity or degree of recurrence of subordinate patterns in both mathematical sequences and empirical time series (33, 34, 39, 41). Precisely, ApEn is a model-free, two-parameter family of statistics: ApEn (m, r), with m a run length, and r, a de facto tolerance width (see Refs. 33 and 41 for practical examples). ApEn parameters of m = 1 and r = 20% of each intraseries standard deviation (SD) were used here for 24-h 10-min data, as previously described for various neurohormone profiles of this length (12, 17, 27, 38, 40, 56, 61, 63). Corresponding values of r were applied for shorter time series, as recently validated (40). ApEn monitors sample-by-sample irregularity and is thus distinguished from conventional analyses of pulsatility, circadian rhythmicity, or short-term (ultradian) periodicity (4, 50, 51, 56, 62). Higher ApEn values denote greater irregularity (or higher process randomness) of repetitive (successive) measurements as reported for the following: GH, ACTH, and prolactin time series in patients with acromegaly, Cushing’s Disease, and prolactinomas compared with age- and sex-matched controls (12, 46, 65); GH patterns
in boys in mid-to-late puberty and in boys and girls after sex-steroid hormone administration (10, 32, 43, 61, 63); GH secretion in females compared with males (13, 38, 61, 63); and ACTH, LH, GH, cortisol, testosterone, and insulin time series in aging vs. younger humans (17, 27, 28, 42, 43, 56, 60).

Statistics

Because the distribution of ApEn values is asymptotically normal (33), ApEn data were compared via the two-tailed Student's t-test with unequal variance to evaluate within- or between-subject contrasts. Primary inferences were confirmed by the nonparametric Wilcoxon signed-rank or rank sum test. Time series were also shuffled (randomly reordered without replacement) 1,000 times to generate a surrogate null distribution of “random ApEn” values.

RESULTS

Figure 1A depicts the impact of continuously infused saline or rh IGF-I on ApEn of 24-h serum GH concentration profiles in healthy young women and men. During saline infusion, mean GH ApEn was higher in women than men ($P < 10^{-3}$), confirming the previous gender distinction in this axis (12, 38, 61, 63). Intravenous rh IGF-I infusion lowered GH ApEn significantly in all seven women ($P = 0.0156$), with an analogous but nonsignificant trend in men ($P = 0.088$), again denoting a sex difference. Lower ApEn values indicate more regular GH release, here attributable to exogenously imposed IGF-I negative feedback. Figure 1B shows the corresponding impact of continuous intravenous infusion of testosterone on LH ApEn in acutely hypocortiogenic men (METHODS). LH ApEn decreased in all six volunteers in response to enforced androgen negative feedback ($P = 0.012$), thus defining more orderly LH secretion patterns.

Figure 2A presents paired GH ApEn values in the fed (and, hence, IGF-I replete) state vs. fasting (IGF-I deprived) context in eight women. Fasting increased GH ApEn in all subjects, identifying greater irregularity of GH secretion ($P = 0.0005$). Figure 2B presents paired LH ApEn values in eight young men adminis-
tered the steroidogenic inhibitor KTCZ (vs. placebo) to withdraw negative feedback by testosterone (64, 67). Hypoandrogenemia increased LH ApEn in seven of eight volunteers ($P = 0.0062$).

Figure 3A depicts paired GH ApEn values in men infused with pulses of GHRH or saline intravenously every 90 min for 3 days. Exogenous GHRH drive augmented GH ApEn in 18 of 19 volunteers ($P < 10^{-4}$). Analogously, Fig. 3B gives paired LH ApEn values in volunteers infused with fixed pulses of GnRH or saline intravenously at 90-min intervals for 14 days. The exogenous GnRH “clamp” elevated LH ApEn in all five subjects ($P = 0.036$).

Figure 4A reports calculated GH ApEn values during intravenous infusion of saline or a selective GHRH-receptor antagonist peptide. Serum GH concentrations during the last 6 h of sampling fell by $\sim 60$–$85\%$ during antagonist infusion. Neither of two statistical expressions for GH ApEn (mean observed ApEn or mean ratio of observed-to-random ApEn) changed significantly. Continuous intravenous infusion of somatostatin, a negative feedback signal on GH secretion, suppressed GH output by a mean ($\pm$SE) of $89 \pm 6\%$ and lowered GH ApEn in 8 of 10 postmenopausal women ($P = 0.011$) (Fig. 4B). Disabling GnRH feedforward input by prior injection of a GnRH agonist (leuprolide) compared with saline yielded a higher mean LH ApEn ($P = 0.0052$) and elevated the ratio of observed-to-random LH ApEn ($P < 10^{-5}$) (Fig. 4C).

**DISCUSSION**

The present interventional analyses show that experimental reduction of IGF-I negative feedback increases the serial irregularity of GH release. Likewise, muting of testosterone negative feedback increases the irregularity of sample-by-sample LH secretion. Conversely, imposition of the foregoing axis-relevant feedback signals (e.g., by continuous intravenous infusion of IGF-I or testosterone) consistently reduced the irregularity of GH and LH output patterns. In aggregate, for the 30 subjects studied in four experiments, the sensitivity of ApEn to detecting a negative feedback perturbation, whether damped or heightened,
was 97%. Accordingly, we infer that negative feedback signaling strength within the GH-IGF-I and LH-testosterone axes strongly governs the quantifiable regularity of hormone secretory subpatterns.

The impact of altered feedforward drive on the orderliness of hormone secretion patterns was studied conversely; i.e., by imposing fixed GHRH and GnRH input stimuli and by opposing endogenous GHRH and GnRH actions. In the first experimental paradigm of enforced “overdrive,” unvarying exogenous GHRH and GnRH pulses consistently increased ApEn (i.e., degraded the regularity) of the resultant GH and LH secretory output profiles. In the 24 subjects studied, the sensitivity of ApEn to detecting predicted regular-
ity changes was 96%. In the second experimental paradigm of blunted GHRH and GnRH feedforward, administration of a downregulating GnRH-receptor agonist, albeit not infusion of an antagonist of the GHRH receptor, markedly attenuated the orderliness of subsequent LH release \( (P = 0.0052) \). Accordingly, three of the foregoing perturbation models that supplant endogenous adaptive signaling indicate that both externally fixed feedforward inputs and antagonism of intrinsic feedforward drive disrupt the regularity of GH and LH secretion.

The notion of feedback-dependent control of the subordinate pattern regularity of neurohormone time series was foreshadowed in an earlier analysis of the thyrotropin (TSH)-thyroidal axis. In this study, increased irregularity of 24-h serum TSH concentration profiles in hypothyroid men was reversed by imposing axis-specific negative feedback with L-thyroxine \( (P = 0.013) \) (58). In analogy, in the salt-sensitive renin-angiotensin system, short-term sodium restriction induced, whereas acute salt excess repressed, irregularity in 24-h renin secretory patterns in 11 of 12 time series so studied [unpublished analysis (52)]. In a third neuroendocrine context, primary gonadal failure elevated ApEn of 24-h follicle-stimulating hormone (FSH) release, whereas testosterone repletion normalized disorderly FSH output in all six men (59). Accordingly, ApEn analyses of a variety of distinct neuroendocrine feedback models (i.e., the GH, LH, TSH, renin, and FSH axes) indicate that the (quantifiable) reproducibility of serial hormone release patterns mirrors axis-relevant positive and negative feedback signal strength with high sensitivity (96–100%).

If the foregoing (11 separate) observations are correct and generalizable, then the apparent failure of a selective GHRH-receptor antagonist to modify GH ApEn (given significant suppression of GH release) could indicate that other nonGHRH agonistic or antagonistic signals maintain the subpattern regularity of GH secretion. In relation to other agonists, one plausible GH cosecretagogue is the recently cloned GHRH-releasing peptide Ghrelin (14, 25). As a pertinent GHRH antagonist, somatostatin is likely to be the critical inhibitory signal. Indeed, somatostatin infusions markedly enhanced GH regularity (Fig. 4B), consistent with a model wherein negative feedback augments orderliness. However, we cannot fully exclude incomplete suppression of endogenous GHRH drive achieved by this dose or schedule of administration of the GHRH-receptor antagonist. The last consideration would be consistent with a recent analysis of ApEn after high-sensitivity immunofluorescence assay of 24-h GH profiles in two siblings harboring a profound loss-of-function (7th intron splicing junction) mutation of the GHRH receptor. Both patients exhibited markedly elevated GH ApEn values (namely, each 5 SDs or \( P < 10^{-6} \) vs. gender, body mass index, and age-matched controls) (F. Roelfsema, J. D. Veldhuis, unpublished data).

The current experiments highlight the utility of a statistically validated regularity measure to monitor variations in the strength of neuroendocrine axis-relevant feedback and feedforward control signals. Whether this inference is applicable to other integrative physiological systems is not established. However, in the stress-adaptive corticotropic axis, glucocorticoid feedback signal intensity strongly modulates the sample-by-sample orderliness of ACTH secretion. Specifically, metyrapone and KTCZ induced hypocortisolemia-stimulated ACTH secretion by 8- to 35-fold and reduced ApEn of the resultant ACTH release profiles in all 23 subjects (16, 57). The fall in ApEn signifies greater regularity of corticotropin release during relief of glucocorticoid negative feedback. The direction of this ApEn change contrasts with that in the GH, LH, TSH, renin, and FSH axes. Although the mechanistic basis for this neuroregulatory distinction is not known, it is not attributable simply to the large magnitude of stimulated ACTH secretory output, because the ApEn metric in mathematical terms is largely scale invariant and translation independent (METHODS). The orderliness of parathyroid hormone (PTH) secretion is likewise enhanced by withdrawing negative feedback, in this case by lowering the ionized calcium concentration, and conversely (47–49). On the basis of these several findings, we speculate that the unique multivalent feedforward and feedback properties of any given adaptive biological network confer their direction of regularity change. For example, the corticotropin axis is regulated by dual repressive actions of adrenal cortisol on hypothalamic and pituitary sites and complex feedforward synergy of hypothalamic arginine vasopressin and corticotropin-releasing hormone on corticotrope-ACTH secretion (7, 24, 26, 44). Precisely which dynamic network properties of this homeostatic system explicate its directionally specific regularity features are not yet evident. In any case, for both the ACTH and PTH axes, a quantifiable alteration in the orderliness of serial hormone output consistently monitors a change in cognate feedback signal strength, identified in an axis-specific direction.

Theoretical formulations of two- or three-parameter-coupled mathematical systems indicate that relative nodal isolation, or greater autonomy of variables in feedback-linked equations, enhances the orderliness of numerical outputs (34, 41). Such reductionist models thematically mirror the foregoing findings in the ACTH and PTH axes, wherein feedback withdrawal (i.e., reduced parameter coupling) facilitates time series regularity. By extension, our observations across multiple axes are consistent with the more general hypothesis that the particular structural linkages within an adaptive biological network further specify the directionality of ApEn changes induced by feedback and/or feedforward modulation (37). Additional intervention experiments and more refined network-based models of multivalued neurohormone ensembles will be required to verify this notion and to clarify the particular mechanistic basis of system-specific diversity of homeostatic control. For example, one simplified stochastic differential equation construct of the multicycoupled GnRH-LH-testosterone feedback/feedforward
axis predicted that withdrawal of androgen-negative feedback on GnRH and LH signaling outputs would increase the irregularity of LH and testosterone secretion, as observed empirically in healthy aging men and experimentally in KTCZ-treated (androgen withdrawn) young individuals (20–23, 29, 30, 42; D. M. Keenan, J. D. Veldhuis, unpublished observations).

In summary, the present perturbation experiments and regularity analyses demonstrate that axis-specific feedback or feedback activity closely supervises the orderliness of resultant secretory patterns in several distinct neuroendocrine contexts. Comparisons of different hormone axes (DISCUSSION) would further suggest that the unique algorithmic structure of an adaptive neuroregulatory network specifies the actual direction of the regularity change. According to this interpretation, we hypothesize that the quantifiable orderliness of a biological time series monitors changes in within-axis signaling strength with the regularity directional preference dictated by the specialized integrative properties of the system. Further exploration of this emergent concept may aid in clarifying the mechanistic basis of selected physiological adaptations and pathophysiological perturbations in feedback and/or feedforward control in complex neurohormone networks.

Perspectives

In several rather different closed mathematical model systems (e.g., coupled logistic, autoregressive moving-average, and composite stochastic-deterministic equations), uncoupling of parameter interactions consistently increases ApEn of the simulated output signal (34, 41). In such more tractable theoretical networks, higher-series ApEn (and, hence, greater irregularity) denotes erosion of feedback control. Conversely, in these simplified model forms, lower ApEn (greater series orderliness) identifies higher-parameter coordination due to stronger negative feedback loops. The present in vivo interventional analyses corroborate such reductionistic inferences in a variety of highly complex neuroendocrine systems; i.e., the GH, LH, TSH, renin FSH, ACTH, and PTH axes. In particular, we show that altered positive or negative feedback signaling modifies ApEn consistently in an axis-specific manner. The axis-relevant directionality of the ApEn change suggests to us that the unique adaptive control properties of any particular physiological system govern the relevant direction of regularity shifts (23, 36). According to this evolving perspective, more detailed analyses of the dynamic features of feedback and feedforward-interlinked axes should advance our mechanistic understanding of the basis for systemspecific homeostatic control. New insights in this challenging arena should also help to clarify the nature of the physiological factors that drive, as well as the putative pathophysiological processes that disrupt, complicated composite stochastic and deterministic systems in health and disease.

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