

Joint pituitary-hypothalamic and intrahypothalamic autofeedback construct of pulsatile growth hormone secretion

Leon S. Farhy¹ and Johannes D. Veldhuis²

¹*Division of Endocrinology and Metabolism, Department of Internal Medicine, School of Medicine, University of Virginia, Charlottesville, Virginia 22908; and* ²*Division of Endocrinology and Metabolism, Department of Internal Medicine, Mayo Medical and Graduate Schools of Medicine, General Clinical Research Center, Mayo Clinic, Rochester, Minnesota 55905*

Submitted 19 February 2003; accepted in final form 10 July 2003

Farhy, Leon S., and Johannes D. Veldhuis. Joint pituitary-hypothalamic and intrahypothalamic autofeedback construct of pulsatile growth hormone secretion. *Am J Physiol Regul Integr Comp Physiol* 285: R1240–R1249, 2003. First published July 24, 2003; 10.1152/ajpregu.00086.2003.—Growth hormone (GH) secretion is vividly pulsatile in all mammalian species studied. In a simplified model, self-renewable GH pulsatility can be reproduced by assuming individual, reversible, time-delayed, and threshold-sensitive hypothalamic outflow of GH-releasing hormone (GHRH) and GH release-inhibiting hormone (somatostatin; SRIF). However, this basic concept fails to explicate an array of new experimental observations. Accordingly, here we formulate and implement a novel fourfold ensemble construct, wherein 1) systemic GH pulses stimulate long-latency, concentration-dependent secretion of periventricular-nuclear SRIF, thereby initially quenching and then releasing multiphasic GH volleys (recurrent every 3–3.5 h); 2) SRIF delivered to the anterior pituitary gland competitively antagonizes exocytotic release, but not synthesis, of GH during interval intervals; 3) arcuate-nucleus GHRH pulses drive the synthesis and accumulation of GH in saturable somatotrope stores; and 4) a purely intrahypothalamic mechanism sustains high-frequency GH pulses (intervals of 30–60 min) within a volley, assuming short-latency reciprocal coupling between GHRH and SRIF neurons (stimulatory direction) and SRIF and GHRH neurons (inhibitory direction). This two-oscillator formulation explicates (but does not prove) 1) the GHRH-sensitizing action of prior SRIF exposure; 2) a three-site (intrahypothalamic, hypothalamo-pituitary, and somatotrope GH store dependent) mechanism driving rebound-like GH secretion after SRIF withdrawal in the male; 3) an obligatory role for pituitary GH stores in representing rebound GH release in the female; 4) greater irregularity of SRIF than GH release profiles; and 5) a basis for the paradoxical GH-inhibiting action of centrally delivered GHRH.

feedback; mathematical model; somatotropic axis; hormone pulsatility; somatostatin; growth hormone-releasing hormone; hypothalamus

THE SECRETION OF GROWTH HORMONE (GH) is pulsatile in the human, monkey, sheep, swine, guinea pig, rat, and mouse (10, 20, 21, 30, 34, 37, 40, 48, 49). In the rodent,

episodic GH release directs somatic growth, cellular gene expression, and autonegative feedback (3, 20, 31, 34, 37, 42). A distinctive feature of pulsatile GH secretion is the dual representation of 1) multiburst volleys, which recur every 3–3.5 h in the adult male rat and every 1.5–2.5 h in men and women, and 2) rapid discrete GH pulses, which arise every 30–60 min within an individual volley (19–22, 30, 37, 38, 42, 48, 49). Such complex patterns of GH release are evident by high-frequency (0.5- and 5-min) sampling paradigms in the unanesthetized rat, sheep, and healthy human (19, 22, 23, 37, 38, 42, 49). The mechanisms that generate composite low-frequency volleys and high-frequency GH bursts are not established.

Our earlier construction of a regulatory basis for combined GH volleys and discrete GH pulses incorporated prompt autofeedback by secreted GH on GH-releasing hormone (GHRH) outflow (thereby eliciting rapidly recurrent events) and delayed feedforward by GH on somatostatin (SRIF) release (thus terminating a volley). In essence, this notion defines two systemic-hypothalamic oscillators with distinguishable sensitivities and periodicities (14). The resultant GH secretory pattern is self-renewing, emulates that of the adult male rat, and is adaptable to female dynamics by gender-specific relaxation of GH-induced SRIF release (15). However, the purely systemic-central nervous system (CNS) feedback structure fails to account for other fundamental experimental observations. The latter include 1) continued GH pulsatility under constant systemic GHRH stimulation, 2) a paradoxical suppressive effect of central GHRH action, and 3) peripheral SRIF withdrawal-induced rebound-like secretion of GH in the adult female rat (13, 20, 27, 30, 34, 42).

The present work formulates and implements an alternative model of GH autoregulation, which combines four distinct mechanisms: 1) long-loop, time-delayed stimulation of SRIF release by blood-borne GH (systemic-CNS control); 2) periventricular SRIF-dependent inhibition of pituitary GH release but not synthesis (CNS-pituitary regulation); 3) arcuate-nu-

Address for reprint requests and other correspondence: J. D. Veldhuis, Division of Endocrinology and Metabolism, Dept. of Internal Medicine, Mayo Medical and Graduate Schools of Medicine, General Clinical Research Center, Mayo Clinic, Rochester, MN 55905 (E-mail: veldhuis.johannes@mayo.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

cleus GHRH-stimulated somatotrope GH synthesis and storage in a releasable pool (hypothalamo-pituitary pathway); and 4) short-loop, rapid, and reciprocal signaling within the hypothalamus between GHRH and SRIF, which sustains GHRH/GH pulse renewal. This construct forecasts self-regenerating GH oscillations within a volley, illustrates a mechanism for GH pulsatility in the face of continuous GHRH drive, and explicates post-SRIF rebound GH secretion in the female.

METHODS

Core GH network. The core ensemble comprises seven principal feedforward and feedback linkages among GH, GHRH, and SRIF (Fig. 1). At any given moment, GH output reflects these primary physiological interactions, which are transduced by implicit effector dose-response interface functions. Individual peptide elimination rates are assumed to be distinct and stable. The primary network-like connections (and their experimental derivation) are 1) GHRH's acute stimulation of GH release from somatotrope cells (8, 18, 26, 30, 37, 42, 43, 45, 46), 2) SRIF's antagonism of GHRH-induced GH secretion (18, 20, 34, 42), 3) GH's delayed feedforward on SRIF release (6, 9, 29, 31, 34, 35, 37), 4) SRIF's inhibition of GHRH secretion (8, 12, 16, 24, 30), 5) GH's repression of GHRH outflow (7, 9, 16, 31, 32), 6) GHRH's time-lagged induction of pituitary SRIF secretion (1, 2, 11, 24, 27, 29, 32–34, 37, 46), and 7) GHRH's induction of the de novo synthesis and accumulation of (releasable) GH in the pituitary gland (20, 34, 42, 45, 46). This ensemble formulation extends an earlier basic construct by including intrahypothalamic GHRH-evoked SRIF outflow and GHRH-stimu-

lated synthesis and accumulation of GH stores (see DISCUSSION). The interconnections defined by the above interfaces (nos. 1–7) yield two coupled oscillators: an intrahypothalamic GHRH-SRIF oscillator (connections 4 and 6 above) and a pituitary-hypothalamic GH-SRIF oscillator (driven via linkage 3).

The foregoing bipartite model provides a platform to examine the present new postulates that a putative CNS GHRH-SRIF oscillator will yield a high frequency of self-sustaining GH pulses within a volley, and the systemic-hypothalamic GH-SRIF oscillator will confer infrequent multiphasic volleys. Concomitantly, we tested GH pulse automaticity under (simulated) continuous GHRH infusion and the contribution of GHRH-enhanced (releasable) pituitary GH stores to postSRIF rebound-like GH secretion.

Connectivity is encapsulated in the following core equations. The prime notation denotes the time derivative (or rate of change of concentration) under feedback and feedforward inputs

$$GH' = -k_1GH + \alpha k_{r,1} \times \left[\frac{(Pool/t_0)^{n_0}}{(Pool/t_0)^{n_0} + 1} \frac{(GHRH/t_1)^{n_1}}{(GHRH/t_1)^{n_1} + 1} \frac{1}{(SRIF/t_2)^{n_2} + 1} \right] \quad (1)$$

$$SRIF' = -k_2SRIF + S_{\min} + k_{r,2,gh} \frac{GH(t-D)/t_3}{1 + GH(t-D)/t_3} + k_{r,2,ghrh} \frac{[GHRH(t-T)/t_6]^{n_6}}{[GHRH(t-T)/t_6]^{n_6} + 1} \quad (2)$$

$$GHRH' = -k_3GHRH + k_{r,3} \left[\frac{1}{(SRIF/t_4)^{n_4} + 1} \frac{1}{(GH/t_5)^{n_5} + 1} \right] \quad (3)$$

$$Pool' = k_{r,4} \frac{(GHRH/t_7)^{n_7}}{(GHRH/t_7)^{n_7} + 1} (M - Pool) - k_{r,1} \times \left[\frac{(Pool/t_0)^{n_0}}{(Pool/t_0)^{n_0} + 1} \frac{(GHRH/t_1)^{n_1}}{(GHRH/t_1)^{n_1} + 1} \frac{1}{(SRIF/t_2)^{n_2} + 1} \right] \quad (4)$$

GH, *SRIF*, and *GHRH* (italicized) signify the concentration of each peptide; *Pool* defines the GH concentration in releasable pituitary stores; *M* is the maximal attainable GH concentration in the pool; *S_{min}* designates minimal baseline "tonic" SRIF release; α is a scaling constant to relate the distribution volumes of the pituitary and systemic circulation; k_1 , k_2 , and k_3 are rate constants of peptide elimination; and $k_{r,1}$, $k_{r,2,gh}$, $k_{r,2,ghrh}$, $k_{r,3}$, and $k_{r,4}$ are rate constants of release or synthesis. n_0 , n_1 , n_2 , n_4 , n_5 , n_6 , and n_7 and t_0 , t_1 , t_2 , t_4 , t_5 , t_6 , and t_7 are Hill coefficients and thresholds, respectively, for the dose-response interfaces numbered in Fig. 1. *D* is the time delay for GH's feedback on SRIF, *T* is the time-delay for GHRH's feedforward on SRIF, and t_3 is the Michaelis-Menten constant defining sensitivity of SRIF feedback on GH.

In this formulation, the dynamics of the GH pool depend simultaneously on time-varying synthesis and release of GH. Algebraically, synthesis is represented by the first term at right in Eq. 4. Synthesis (but not release) of GH is stimulated by GHRH (through the corresponding Hill function), even in the presence of SRIF competition, and depressed as the pool approaches saturation (the term " $M - Pool$ " in Eq. 4). Pool saturation denotes that the maximal GH concentration attainable remains below a certain asymptotic limit (the constant *M*). Actual GH release is described by the second term at right in Eq. 4. Release is antagonized by SRIF and stimulated by GHRH depending on GH pool size.

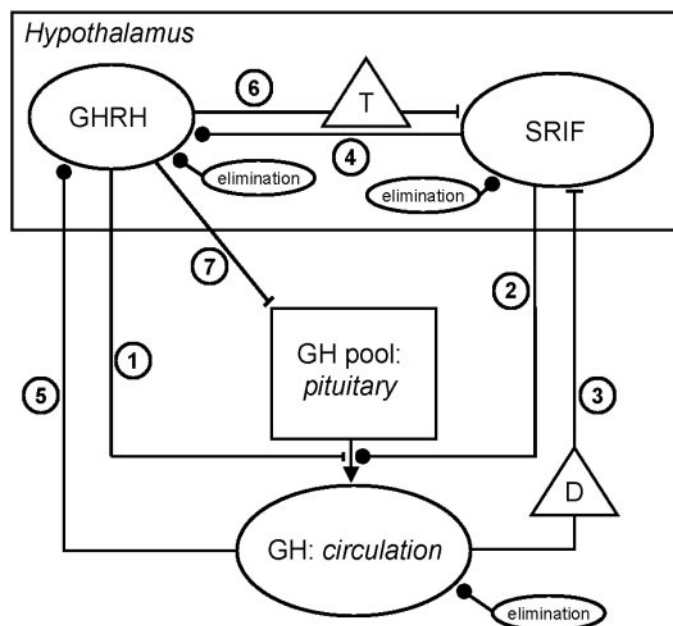


Fig. 1. Schema of primary feedback connections linking core hypothalamic centers, the anterior pituitary gland and the systemic circulation. Inhibitory interfaces and elimination processes are denoted by lines terminating in solid circles; stimulatory interactions are defined by T endings. Formalized interactions are identified by circled Arabic numerals 1–7; triangles containing T and D denote the time delays in the corresponding relationships (see METHODS). GH, growth hormone; GHRH, GH-releasing hormone; SRIF, somatostatin.

Deterministic features. The following assumptions are implicit in the interactive network represented algebraically by Eqs. 1–4. 1) A pulse of GH requires all three of the following: releasable GH stores, stimulation by GHRH, and relative relief of SRIF restraint (designated in the term in brace parentheses after $k_{r,1}$ in Eq. 1). 2) GH and GHRH each stimulate SRIF release (defined by the terms after $k_{r,2,gh}$ and $k_{r,2,ghrh}$, respectively, in Eq. 2). 3) GHRH secretion proceeds under combined withdrawal of GH feedback and relief of SRIF inhibition (given in the term after $k_{r,3}$ in Eq. 3). 4) The releasable pituitary GH pool is depleted partially and saturated fully (parameter M in Eq. 4). 5) GHRH-driven synthesis of GH expands the releasable pool in inverse proportion to nearness to saturation (multiplier fraction after $k_{r,4}$ in Eq. 4). And 6) GH- and GHRH-stimulated SRIF outflow suppresses GHRH and GH secretion (Eqs. 1 and 3), i.e., SRIF inhibits both the hypothalamus and pituitary gland.

GHRH's drive of SRIF release is stated for notational simplicity as proportionate to GHRH concentration (Eq. 2). Any of numerous other neurotransmitters that mediate GHRHergic transsynaptic control, if relevant, would replace GHRH in acting on internuncial and/or SRIFergic neurons.

Parameter definition. A detailed justification of the choice of interface parameters is given in Refs. 14 and 15. Incomplete experimental data require indirect estimation of kinetics for the unobserved pituitary GH pool: t_0 , n_0 , α , $k_{r,4}$, t_7 , n_7 , and M . A priori criteria are pulsatile GH release without store exhaustion and a threshold t_5 that ensures concentration-dependent suppression of GHRH secretion by GH. Table 1 shows parameters used in the present analyses.

Justification of accumulation of pituitary GH stores. In vitro and in vivo SRIF blocks the release, but not GHRH-stimulated synthesis and accumulation, of GH in somatotrope cells (18, 20, 35, 45, 46). Equations 1 and 4 incorporate this precept by allowing GHRH to promote the (saturable) accumulation of releasable GH stores when SRIF is present. Second, we test the implications of an allowance that systemic SRIF suppresses GHRH release in the arcuate nucleus (see DISCUSSION).

Rebound GH release. The network connectivity of Fig. 1 permits an evaluation of the impact of feedback-dependent unleashing of acute GH secretion by sequential exposure to and withdrawal of systemic SRIF. We assess the conse-

quences of SRIF to block GH release but not inhibit GHRH-dependent synthesis and accumulation of GH stores (20). The primary postulate is that post-SRIF rebound secretion of GH arises by way of increased GH stores (due to SRIFergic inhibition of GH release despite ongoing GH synthesis) and elevated GHRH release (due to decreased hypothalamic SRIF outflow associated with reduced GH availability for feedback) (6, 7, 16, 20, 28, 32, 37). A complementary prediction is that hypothalamic GHRH secretion during peripheral SRIF infusion would remain pulsatile, unless circulating SRIF can enter the CNS and fully suppress GHRH release (12).

Continuous infusion of GHRH. The macromolecules injected intravenously accumulate promptly in the median eminence and arcuate nucleus but not in the periventricular nucleus (20, 34). Thus we compare the impact of constant systemic GHRH stimulation under the assumption of either no or variable access of infused GHRH to SRIF neurons. A truncated transcript of the GHRH gene is expressed in the foregoing two hypothalamic nuclei, as quantitated by RT-PCR, sequencing of the cDNA product, and Northern blot hybridization (47); and, central delivery of synthetic GHRH stimulates SRIF release acutely in vivo in the adult male rat and in vitro from incubated fragments of the median eminence (1, 2, 11, 24, 27, 29, 32–34, 37).

Simulation of SRIF or GHRH infusion. To simulate systemic or central delivery of SRIF or GHRH, we define the changing concentration of the infused substance as

$$C' = -kC + \text{Inf} \quad (5)$$

where C is the time-varying predicted and instantaneous concentration of the exogenous peptide (GHRH or SRIF), k is the corresponding elimination rate constant, and Inf is the peptide infusion rate. The (infused) C term is additive to the endogenous concentration term, *SRIF* or *GHRH*, in Eqs. 1–4, solely if exogenous peptide gains access to the particular endogenous peptide pool (e.g., if exogenous SRIF comingles with hypothalamic SRIF).

RESULTS

Basic model output. Figure 2 depicts simulated (reference) profiles of time-varying concentrations of GH, SRIF, and GHRH (Fig. 2A) and releasable pituitary

Table 1. Nominal parameter set applied in male rat reference model

	Elimination Rate Constant	Release or Interface Constant	Threshold Value	Hill Coefficient
GH	$k_1 = 2.7 \text{ h}^{-1}$	$k_{r,1} = 17,325 \text{ ng} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$	$t_5 = 1,125 \text{ ng/ml}$	$n_5 = 2$
SRIF	$k_2 = 5 \text{ h}^{-1}$	$k_{r,2,gh} = 4,200 \text{ pg} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$ $k_{r,2,ghrh} = 1,400 \text{ pg} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$ $S_{\min} = 143 \text{ pg} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$	$t_2 = 35 \text{ pg/ml}$ $t_4 = 22 \text{ pg/ml}$	$n_2 = 3.5$ $n_4 = 3.5$
GHRH	$k_3 = 8 \text{ h}^{-1}$	$k_{r,3} = 92,160 \text{ pg} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$	$t_1 = 1,966 \text{ pg/ml}$ $t_6 = 1,966 \text{ ng/ml}$ $t_7 = 400 \text{ pg/ml}$	$n_1 = 3.5$ $n_6 = 3.5$ $n_7 = 2$
GH pool		$k_{r,4} = 0.45 \text{ ng} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$ Maximal pool concn scaling constant $\alpha = 1.125$	$t_0 = 533 \text{ ng/ml}$ $M = 2,667 \text{ ng/ml}$	$n_0 = 3$
Time-delay constant $D = 1.2 \text{ h}$; $T = 0.3 \text{ h}$			Michaelis-Menten constant $t_3 = 2,626 \text{ ng/ml}$	

Elimination rate constant, decay half-life = $\ln 2/k_i$ ($i = 1, 2, 3$); release or interface constant, rate of change of induced secretory response; threshold value, input (effector) concentration (concn) above or below which slope of dose-response function begins to change rapidly; Hill coefficient, steepness term for dose-response function; S_{\min} , determines basal (minimal) rate of somatostatin (SRIF) secretion; time-delay constant, time delay for growth hormone (GH) feedback on SRIF = D and time delay for GH-releasing hormone (GHRH) feedback on SRIF = T ; Michaelis-Menten constant, for GH-induced stimulation (feedforward) of SRIF outflow. See METHODS for further explanation.

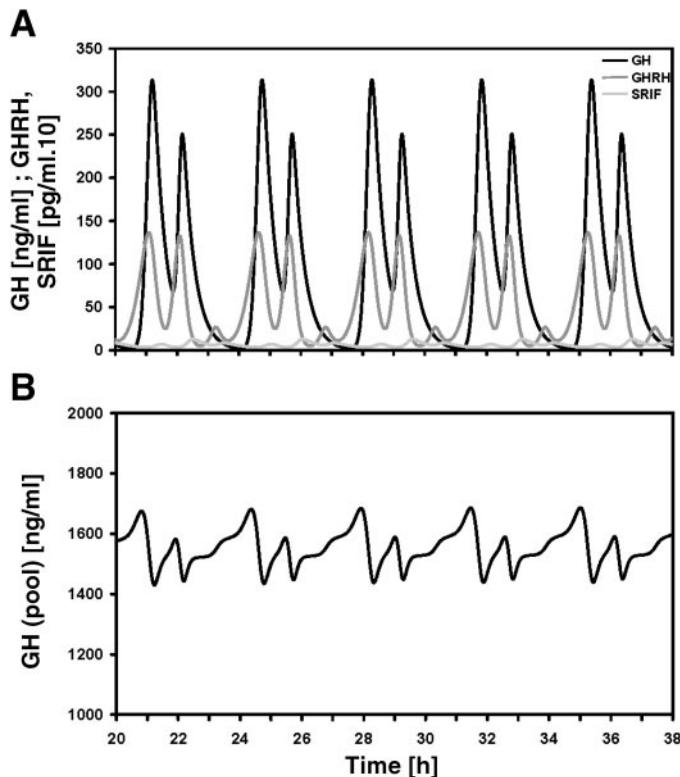


Fig. 2. *A*: simulated time series driven by the reference (male) model (see Table 1 and Fig. 1). Plots are simultaneously evolving SRIF, GHRH, and GH concentrations generated every 30 s by the network shown in Fig. 1 and then discretized to 5-min intervals. *B*: changing relative size of GHRH-releasable pituitary GH pool (arbitrary maximum). Time series were allowed to unfold for 20 h before the indicated 18-h record.

GH stores (Fig. 2*B*) in the adult male model. In the present formulation, successive GH pulses evolve as follows. GHRH is released into portal blood during relatively permissive outflow of hypothalamic SRIF. GHRH stimulates prompt GH secretion from releasable pituitary stores, as GHRH-on-GH dose-responsive stimulation of somatotropes traverses the steeply ascending portion of the agonist-response interface, defined by the action threshold (t_1). Concomitantly, GHRH feedforward begins to augment de novo synthesis of GH. Neuronal GHRHergic activity (denoted algebraically by the rising GHRH concentration; Eq. 3) evokes central SRIF release after a short time delay T . The latency, T , is required for GHRH to attain the dose-response threshold t_6 of strong feedforward drive. As a result, SRIFergic outflow increases toward the cognate inhibitory threshold (t_4), whereby arcuate-nucleus GHRH secretion is repressed. Concurrently, SRIF secreted to the pituitary gland antagonizes the release, but not synthesis or storage, of GH by somatotrope cells. Attendant elimination of GHRH peptide (by diffusion and distribution into the systemic circulation and cerebrospinal fluid and irreversible metabolism) contributes to ending the first GH secretory peak within a volley. Attenuation of GHRHergic drive withdraws the latter intrahypothalamic stimulus to SRIF secretion. Subsequent rapid elimination of SRIF

from the periventricular SRIFergic synaptic contacts made with arcuate-nucleus GHRH neurons disinhibits GHRH release, thus initiating another GHRH pulse that continues the multiburst secretory volley. Recurrent cycling of GHRH and SRIF with phase disparity allows somatotrope accumulation of GH stores (Fig. 2). Accumulation proceeds when GHRH signaling, GH gene transcription, and/or mRNA stability maintain GH biosynthesis and SRIFergic effects inhibit somatotrope exocytosis.

The foregoing formulation illustrates that reciprocal interactions between hypothalamic GHRH and SRIF could sustain recurrent GHRH and GH peaks within a volley. The volley unfolds until GH concentrations increase sufficiently in the circulation and hypothalamus to stimulate release of periventricular SRIF after a distinct time delay D . The resultant outflow of SRIF restricts pituitary GH release and hypothalamic GHRH secretion (5). These twofold actions quench GH and GHRH pulses within a volley. Accordingly, the duration of any given volley reflects the de facto delay required for systemic GH-hypothalamic SRIF feedback. Concentrations of GH in blood and interneuronal fluids decline at a slow rate. The attendant delay enforces prolonged interval release of SRIF. The latter interval exceeds intravolley recovery times, which would mirror interneuronal synaptic (reciprocal SRIF-GHRH) signaling latencies. GH stores accumulate so long as release and/or pituitary effects of GHRH persist in stimulating GH synthesis in the transitional intervals, when 1) GH induces SRIF outflow by autofeedback, but has not yet inhibited GHRH (ending a volley); and 2) SRIF outflow wanes releasing restraint on GHRH (initiating a new volley; Fig. 2*B*).

Simulations further illustrate that high-frequency GHRH-SRIF oscillations expressly require GHRH stimulation of SRIF. Depletion of this pathway leaves low-frequency (~ 3 -h) pulses driven by the long-latency GH-SRIF oscillator. High-frequency GH oscillations also disappear if $T = 0$ (time delay for GHRH to stimulate SRIF outflow), since instantaneous SRIF release damps the intrahypothalamic GHRH-SRIF oscillator. Thus feedback delay in this model is obligatory to sustain rapid GHRH/GH pulsatility within a volley.

Theoretical considerations and empirical observations indicate that feedback connections within an interlinked network control the serial orderliness (or sequential regularity) of output patterns (39, 41). One objective ensemble regularity measure is the approximate entropy (ApEn) statistic, as justified earlier to compare the relative orderliness of limited time series and thereby quantitate the relative admixture of deterministic and stochastic processes (19, 38). Table 2 gives ApEn and normalized ApEn ratio for simulated (noise-free) GH, GHRH, and SRIF release profiles in the adult male model. For ApEn normalization, each time series is shuffled randomly 200 times to generate a distribution of random (null) ApEn (50, 52). Normalized ApEn is defined here as the mean ratio of observed ApEn (a single value per series) to each of 200 null ApEn values. Series are generated by 5-min discreti-

Table 2. Comparative regularity (orderliness) of hormone concns monitored by scale-independent ApEn

	ApEn (m_1)	ApEn (m_1) Ratio
<i>Untransformed time series</i>		
GH	0.5505	0.3150
GHRH	0.6887	0.3961
SRIF	0.8858	0.4648
<i>Rate of change of concn</i>		
GH	0.7139	0.4126
GHRH	1.0786	0.5501
SRIF	1.0784	0.5718

Data are simulated GH, SRIF, and GHRH time series based on the male-like feedback model for a 22-h modeling period. ApEn, approximate entropy statistic; untransformed time series, data are simulated noise free; rate of change of concn, first-differenced time series to detrend epochs in the data; m_1 , pattern recurrence in data pairs; ratio, mean ratio of observed ApEn to null ApEn (ApEn of each of 200 randomly shuffled versions of observed series). See RESULTS for further details.

zation (265 apparent samples over 22 h) of the primary 30-s concentrations. ApEn ratios are cited for simultaneously evolving GH, SRIF, and GHRH concentrations and their first differences (stationarized series). First-differenced ApEn values yield comparable inferences to those for native profiles. ApEn was quantitated for pattern recurrence (template) vector lengths of $m = 1$ and a scalar tolerance (threshold) range of $r = 0.2$ SD, where SD is the standard deviation of the data set (for details, see Refs. 19 and 38). Higher ApEn ratios approaching 1.0 approximate empirically mean random (equivalent to shuffled, null ApEn) and therefore define greater disorderliness (less subpattern reproducibility). Thus SRIF was the most irregular and GH the least irregular, with GHRH exhibiting an intermediate degree of relative orderliness.

SRIF-induced rebound GH release. Systemic SRIF delivery and withdrawal was simulated as described in METHODS (see *Simulation of SRIF or GHRH infusion*) by adding a new Eq. 5 to the core system. The infusion term $\text{Inf}(t)$ differs from zero only during the anticipated infusion period, when it equals $5,000 \text{ pg} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$. The same elimination rate constants are assumed for endogenous and exogenous SRIF. Under a simplifying model assumption that exogenous SRIF does not inhibit GHRH neurons [the "infusion" $C(t)$ (Eq. 5) is added to $\text{SRIF}(t)$ in Eqs. 1 and 4 but not Eq. 3], pulsatile GHRH release continues despite peripheral inhibition of GH output, thereby increasing GH synthesis and storage. Withdrawal of short-term (4-h) SRIF inhibition from the circulation in this model induces prompt rebound-like release of GH (Fig. 3A). The resultant post-SRIF GH secretory burst partially depletes releasable GH stores. Rebound is accompanied by elevated release of GHRH peptide (exemplified in Fig. 3 by a horizontal line passing through the peak GHRH value during the rebound). Elevated GHRH release has been documented in similar context by direct central (hypothalamo-pituitary portal) venous sampling in the awake ram (31).

Figure 3, B and C, illustrates predicted responses to a relatively abbreviated and extended SRIF infusion (2.5 and 7 h compared with 4 h). These simulations forecast that relative expansion of the GH pool augments rebound GH release until pool size approaches saturation asymptotically. The foregoing outcome was modeled by augmenting the contribution of the GH pool to post-SRIF-induced rebound GH release under equivalent GHRH release (2-fold decrease in t_0 , 2-fold increase in $k_{r,1}$, and 2-fold decrease in α to preserve $\alpha k_{r,1}$ unchanged; see METHODS).

Additional simulations show that in this model system, SRIF infusion does not disrupt GHRH pulse timing. Therefore, the magnitude of rebound GH release post-SRIF depends on the quantity of releasable GH stores, concomitant GHRH concentration, and the rates of infusion and elimination of SRIF. Incomplete

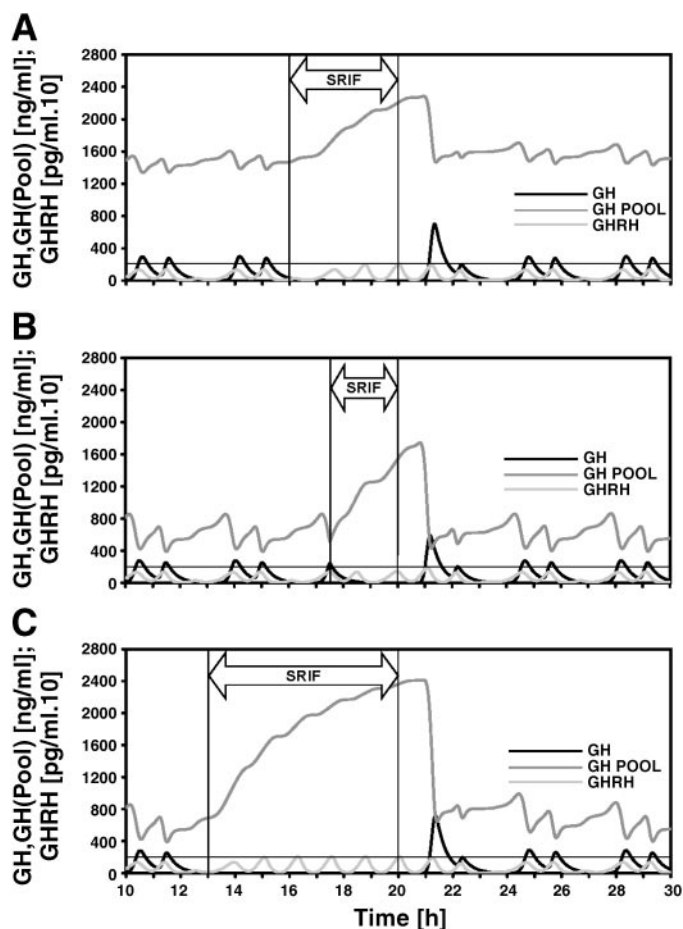


Fig. 3. Predicted effect of sequential infusion and withdrawal of systemic SRIF on GH and GHRH output and the GH pool. A: simulated 4-h SRIF infusion (timeline: 1600–2000) based on the feedback parameters summarized in Table 1. B and C: abbreviated vs. prolonged SRIF infusion (2.5 h in B and 7 h in C) when the contribution of the GH pool is augmented (2-fold decrease in t_0 , 2-fold increase in $k_{r,1}$, and 2-fold decrease in α ; see METHODS). Top curves depict pituitary GH pool size. Cessation of a simulated SRIF infusion (\leftrightarrow) abruptly 1) diminishes GH pool size, 2) initiates rebound-like GH release, and 3) allows responsiveness to GHRH output (B). Horizontal line demarcates elevation in SRIF-induced GHRH release compared with preinfusion GHRH outflow.

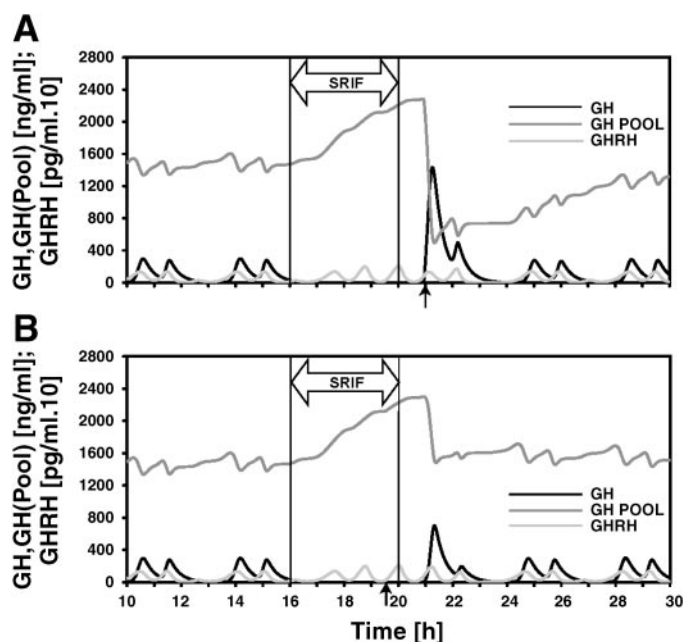


Fig. 4. A: predicted impact of a single GHRH stimulus (arrow on the "Time" axis) administered shortly (20 min) after abrupt interruption of a simulated 4-h infusion of SRIF (1600 to 2000). Rebound-like release of GH is accentuated markedly by an appropriately timed GHRH signal (see Fig. 3A). B: model-based response to a controlled exogenous GHRH pulse (arrow on the "Time" axis) introduced 30 min before withdrawal of a simulated 4-h infusion of SRIF. Rebound amplitude is unchanged compared with the output depicted in Fig. 3A (post-SRIF rebound without a GHRH stimulus).

withdrawal of exogenous SRIF during the first postinfusion GHRH spike would limit maximal GH release. Thus for uniformity, simulations depicted in Fig. 3 illustrate termination of SRIF infusion during a GHRH zenith.

Figure 4A illustrates the predicted effect of a single GHRH stimulus imposed after cessation of a continuous (4-h) infusion of SRIF (with allowance for SRIF elimination). Continuous SRIF infusion was modeled as described, and bolus GHRH was injected by a delimited burst of peak rate of $120,000 \text{ pg} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$. The agonist/antagonist sequence amplifies rebound GH release, as reported in vivo and in vitro for the adult male rat pituitary (8, 20, 34, 46). In contrast, prolonging the (post-SRIF) interval before delivering a GHRH pulse elicits a smaller GH secretory response because of depletion of the releasable GH pool under endogenous GHRH action (not shown). Likewise, delivery of a GHRH stimulus 0.5 h before ending the SRIF infusion is ineffectual (Fig. 4B). These simulations assume that infused GHRH does not stimulate SRIF-producing neurons [the GHRH infusion $C(t)$ (Eq. 5) is added to $GHRH(t)$ in Eqs. 1 and 4 but not Eq. 2]. The latter proviso does not exclude delayed GH drive of SRIF induced by the first peak and expected hypothalamic GHRH stimulation of SRIF.

SRIF withdrawal-induced rebound secretion of GH in the female-like model. To examine post-SRIF-induced rebound release of GH in a female-like construct, we attenuated GH-dependent stimulation of SRIF se-

cretion in the male model. This adaptation is described algebraically in Ref. 15. We then compared the magnitude of post-SRIF rebound-like GH release in the presence (as above) and absence of a time-varying GH pool by replacing the release-control function

$$\frac{(Pool/t_0)^{n_0}}{(Pool/t_0)^{n_0} + 1}$$

with a constant in Eq. 1. Figure 5 illustrates that inclusion of a releasable GH pool allows rebound-like GH secretion in the female-like formulation.

Continuous GHRH infusion. Continuous systemic GHRH delivery was simulated at a rate of $1,600 \text{ pg} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$ with allowance for variable access of exogenous GHRH to SRIF neurons after a finite time delay. For graphical purposes, the 20-h infusion started at 1000 and ended at 3000. Because CNS access of exogenous GHRH is an unknown property that could influence hypothalamo-pituitary responsiveness, we explored the impact on GH output of variable uptake of GHRH into the hypothalamus (Fig. 6). Figure 6, A–D, illustrates outcomes of an assumed relative availability of GHRH to the hypothalamus vis-à-vis anterior pituitary gland of 100 (1:1), 75, 50, and 25%. Unrestricted access of GHRH to SRIF-releasing neurons in this construct represses GH pulse amplitude by delayed feedforward on SRIF. The latter phenomenon is observed after in vivo intracerebroventricular infusion of biosynthetic GHRH in the mature male rat and after in vitro incubation of GHRH with fetal hypothalamic neurons or adult median-eminence neural tissue (1, 2, 11, 27, 29, 32, 33). In further simulations, we observed that the onset of continuous GHRH infusion initially induces high-amplitude GH pulses. Damping of GH

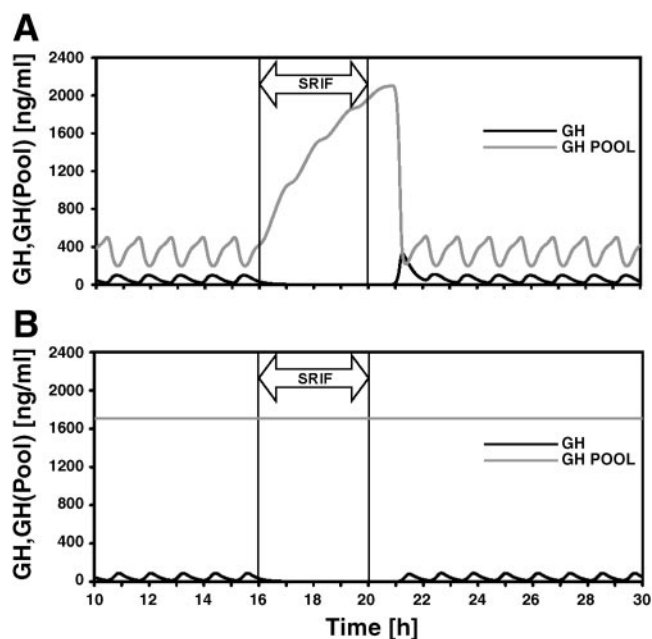


Fig. 5. Dependence of a female-like (GH feedback-muted) construct on a replenishable (A) vs. fixed (B) pituitary GH pool in generating rebound-like GH release at the end of a brief SRIF infusion (\leftrightarrow).

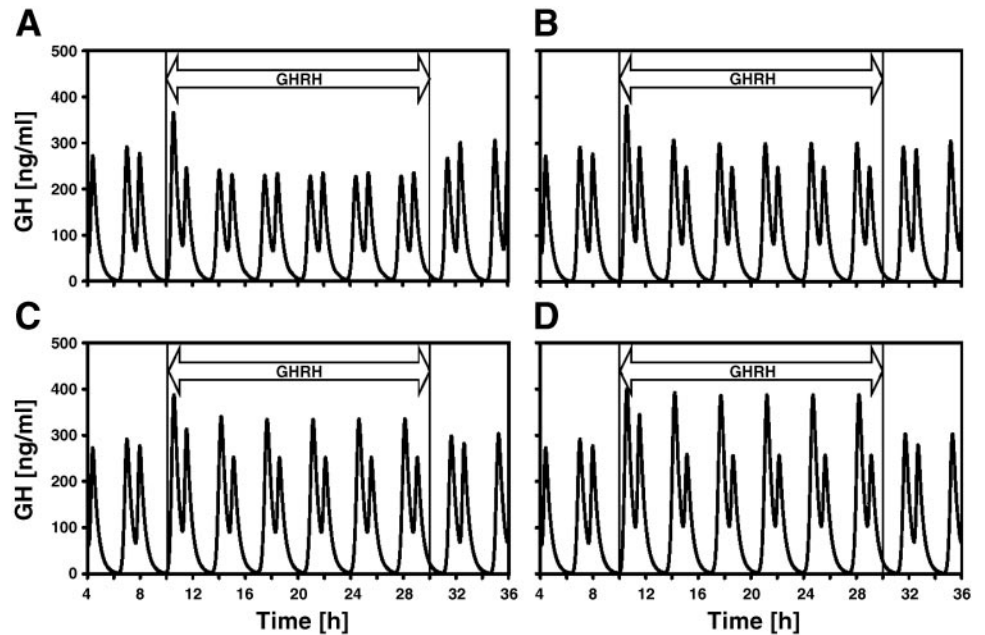


Fig. 6. Predicted outcome of peripherally infused GHRH (1000–3000) under assumptions of hypothalamic uptake of 100 (A), 75 (B), 50 (C), and 25% (D) of circulating peptide. Central actions of GHRH include paradoxical inhibition (100%) and absent stimulation (75%) of GH secretion.

pulse size thereafter reflects the assumed delay in cyclical GHRH-stimulated and systemic GH-induced SRIF release. The sensitivity of suppression of GH secretion to the amount of infused peripheral GHRH was explored by elevating the infusion rate by two- and eightfold (Fig. 7). In this experiment, the hypothalamic uptake of the circulating peptide was assumed to be 100%, and the feedforward latency was prolonged by fourfold to match the delay in GH-on-SRIF drive. The twofold increase in GHRH delivery rate (Fig. 7A) yielded a pseudofeminized GH pattern of low-amplitude pulses (because of secreted GHRH-driven SRIF release) and elevated interpulse concentrations (because of infused GHRH). The eightfold increase in the GHRH infusion rate (Fig. 7B) obliterated GH pulsatility and resulted in rebound-like GH secretion thereafter. The fourfold extended lag time enhanced the magnitude of GHRH dose-dependent rebound. The latter occurred at the predicted time of cyclical GHRH secretion release and was associated with partial depletion of GH stores. Appropriate timing of elevated GHRH release is due to low GH-driven SRIF outflow compared with the GHRH infusion dose.

DISCUSSION

The present analyses demonstrate that reciprocal intrahypothalamic oscillations between SRIF and GHRH provide a mechanistic model that reproduces physiological features of *in vivo* GH release patterns. Specifically, unlike an earlier alternative formulation (14), the present ensemble construct correctly forecasts 1) rapid GH pulses within a multiphasic secretory volley, 2) rebound-like GH release after sequential SRIF exposure and withdrawal, 3) sustained GH pulsatility under continuous (exogenous) GHRH stimulation, 4) accumulation of GHRH-releasable pituitary GH stores, and 5) paradoxical GH-inhibiting action of central GHRH uptake. Each of these regulatory principles is well established experimentally in the rodent, and several are inferable indirectly in the human (20, 25, 34, 37).

Detailed simulations predicted several pivotal regulatory mechanisms. First, saturable accumulation of GHRH-releasable somatotrope stores is crucial to the generation of recurring high-amplitude GH pulses within a multiphasic release episode (volley) in the

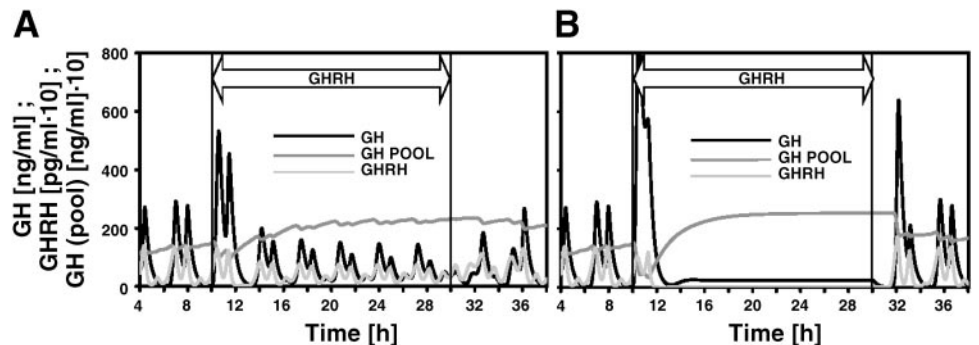


Fig. 7. Sensitivity of suppression of GH secretion to elevation (with respect to the experiment depicted in Fig. 6) of the simulated GHRH infusion rate by 2-fold (A) and 8-fold (B). The feedforward (GHRH-on-SRIF) latency was increased by 4-fold, and the hypothalamic uptake of the circulating peptide was assumed to be 100%.

male model. Indeed, simulated depletion of the saturable pituitary GH pool damps the amplitude of successive GH secretory bursts in an evolving volley. Second, GHRH-stimulated GH synthesis under SRIF-inhibited GH release potentiates post-SRIF-induced GH secretion in the male-like construct. Moreover, the replenishable GH pool model is obligatory to simulate post-SRIF rebound release of GH in the female formulation, wherein GH autofeedback is attenuated. Both projected outcomes agree with experimental data in the adult rat (37, 42, 45, 46). And, third, systemic exposure to SRIF inhibits GH secretion, which in the present construct limits endogenous SRIF restraint and (on SRIF withdrawal) induces rebound-like GH release. Elevated GHRH release has been demonstrated directly by frequent hypothalamo-pituitary portal-venous sampling in the unanesthetized ram and inferred indirectly by passive GHRH immunoneutralization in the adult male rat (8, 30).

Clinical investigations have also unveiled SRIF-induced rebound GH secretion, which in one study was not blocked by administration of a GHRH-receptor antagonist peptide (3, 4, 13, 22, 26, 43, 51). The latter paradoxical outcome could signify a species difference in the post-SRIF rebound mechanism. On the other hand, according to the simulations described here, failure to inhibit rebound GH release in the human administered high concentrations of a GHRH antagonist could denote central stimulatory effect directly on the hypothalamic GHRH-SRIF oscillator. The latter notion follows from extensive experimental data documenting (paradoxical) inhibition of GH secretion via GHRH-specific central neural stimulation of SRIF release (1, 2, 5, 11, 16, 27, 30, 34) and structural evidence of bidirectional GHRH-SRIF connectivity (24, 26). Simulated central (SRIF) access of infused GHRH also paradoxically suppresses pulsatile GH secretion (Fig. 6). On the basis of this representation, (minimal) CNS uptake of a GHRH-receptor antagonist would enhance rather than repress post-SRIF rebound-like GH release by opposing central GHRH drive of SRIF outflow.

The current computer-assisted simulations show that intrahypothalamic oscillations between SRIF and GHRH afford a plausible mechanistic basis for the paradox of unchanged GH pulse frequency during constant GHRH infusion. For example, GH pulsatility clearly persists, but remains unexplained to date, in rare patients with ectopic (tumoral) GHRH production and in healthy individuals given constant intravenous infusion of biosynthetic GHRH (13, 20). Patients harboring a complete inactivating (truncational) mutation of the GHRH-receptor gene also retain a normal daily frequency of GH pulses, albeit of 30-fold reduced amplitude (44). The present analyses forecast a stable GH pulse frequency in the first two contexts, so long as systemic GHRH and GH do not directly restrain the putative hypothalamic SRIF/GHRH oscillator mechanism purported to set pulse timing. The hypothesis that the central GHRH-SRIF oscillator is isolated from systemic GHRH and GH autonegative feedback could be tested expressly by hypothalamo-pituitary portal-

venous sampling of pulsatile GHRH release in the intact awake animal during constant infusion of (heterologous) GHRH and after bolus injection of GH. Important nonexclusive considerations are 1) GHRH neurons stimulate SRIFergic pathways via nonGHRH receptor-dependent neurotransmitters, thus allowing GHRH-SRIF interactions and GH pulse renewal to proceed at a normal frequency in patients with loss-of-function mutations of the GHRH receptor (44); and 2) redundant or collateral signals (such as neuropeptide Y or galanin) maintain GH pulse frequency in the face of excessive or diminished GHRH-dependent oscillations.

In the present model, the inferred descending rank order of ApEn ratios (most irregular to most regular) is $SRIF > GHRH > GH$ for both untransformed and stationarized time series. In our earlier model (14), predicted GH, GHRH, and SRIF profiles emerge from different feedback connectivity, which yields a descending order of ApEn ratios of $GHRH > SRIF > GH$. Available experimental data do not unequivocally distinguish the relative (quantifiable) regularity of SRIF and GHRH release. However, we report greater irregularity of SRIF than GH release using 5-min sampling of cavernous-sinus blood in the conscious unrestrained ewe (SRIF and GH ApEn values were 94 ± 4.3 and $72 \pm 8.1\%$, respectively, of the mean irregularity of 1,000 individual random-shuffled cognate series; $P = 0.034$; Ref. 49).

The current mechanistic formulation of high-frequency GH pulses within multiphasic volleys differs from an earlier notion (14, 15). Specifically, the initial model does not include intrahypothalamic bidirectional coupling between GHRH and SRIF to drive high-frequency pulse renewal. Rather, GH is envisioned to act briefly and reversibly via pituitary secretion to the arcuate-nucleus (36), which suppresses GHRH secretion at a threshold too low to induce SRIF release (14, 15). However, both representations of autoregulation require a longer time delay for systemic GH concentrations to stimulate periventricular SRIF release and quench (otherwise indefinite) continuation of a multiphasic volley (6, 9, 20, 31, 34, 35, 37, 42). SRIF release from nerve terminals in the median eminence terminates a volley by blocking somatotrope exocytosis (7, 9, 12, 16, 20, 24, 30, 32, 34, 37, 42). GH feedback-induced outflow of SRIF into portal blood maintains low intervalley somatotrope secretion until GH concentrations decline by metabolic elimination in the circulation and CNS. Two constructs allow examination of other mechanistic considerations. For example, in future studies, a testable postulate is that central GH drive of periventricular SRIF release may repress putative intrahypothalamic oscillations between GHRH and SRIF. Repression would be relieved by waning GH concentrations, thereby triggering rebound-like GHRH and GH secretion. The foregoing dynamics are concordant with selected biological oscillatory mechanisms that require implicit or explicit involvement of delayed negative feedback (17).

Intervalley (nadir) GH concentrations in the adult male rat are undetectable in most current assays,

consistent with prominent periventricular SRIF secretion induced by GH autofeedback (5, 6, 8, 9, 20, 29, 32). In the female rodent, lesser GH-induced SRIF outflow would unmask activity of the proposed intrahypothalamic GHRH-SRIF oscillator mechanism otherwise made unapparent (albeit present) by pituitary inhibition because of cycles of autofeedback (14, 15, 20, 34, 35, 37). Experimental data show that GH-dependent feedback on SRIF neurons is attenuated, but not abolished, in the adult female rat (15, 19, 20, 31, 34, 37, 38, 42). In the current formulation, partial, rather than complete, muting of GH autoinhibition in the female animal predicts occasional epochs of pluriphasic GH release, as observed under intensive (5-min) and extended (6- to 24-h) monitoring of GH secretion in the female rodent and ruminant (19, 38, 42, 49). A comparable inference of disinhibited GHRH-SRIF oscillatory activity would apply to the nearly continuous train of GH secretory bursts in the presumptively low SRIF milieu associated with fasting or deep sleep in the human (22, 26).

Perspectives

Silencing of CNS SRIF subtype 1-specific receptor function by intracerebroventricular infusion of specific antisense oligodeoxynucleotide represses GH pulse amplitude in the adult male rat (28). Such data demonstrate CNS autoregulation via SRIF receptors but do not establish the particular locus (loci) involved. In principle, topographically specific models of SRIF action could embody primarily arcuate (intra-) nuclear SRIF/GHRH connectivity or periventricular/arcuate (inter-) nuclear SRIF/GHRH linkages (12, 20, 24, 34, 35, 37, 42). For example, in the latter perspective, a pulse of GH would evoke periventricular SRIF release into hypothalamo-pituitary portal blood (thus blocking GH exocytosis) and concomitantly exert SRIFergic transsynaptic inhibition of arcuate-nucleus GHRH neurons (thereby repressing GHRH pulse amplitude). Distinguishing between the foregoing formulations (and possible hybrid models that include each) is not facile. Indeed, interconnectivity may be redundant or complementary, inasmuch as immunoreactive peptidergic neuronal terminals and cognate receptors for GHRH and SRIF are each detected in both the periventricular and the arcuate nucleus (1, 2, 8, 11, 24, 27, 29, 32, 33, 42, 47). In light of the foregoing complex issues, the present analyses underscore the expected complementarity of experimental data and model-assisted predictions to probe physiological regulation.

The authors acknowledge the expert manuscript assistance of J. Plote and the statistical advice of Drs. D. Keenan (Charlottesville, VA) and S. Pincus (Guilford, CT).

DISCLOSURES

This work was supported in part by National Institutes of Health Grants K25-HD-01474 (to L. S. Farhy) and RO1-AG-14799 and AG-19695 (to J. D. Veldhuis) and General Clinical Research Center Award NCRR-M01-00585 (to Mayo Clinic and Foundation).

REFERENCES

1. **Aguila MC, Boggaram V, and McCann SM.** Insulin-like growth factor I modulates hypothalamic somatostatin through a growth hormone releasing factor increase in somatostatin release and messenger ribonucleic acid levels. *Brain Res* 625: 213–218, 1993.
2. **Aguila MC and McCann SM.** Stimulation of somatostatin release in vitro by synthetic human growth hormone-releasing factor by a nondopaminergic mechanism. *Endocrinology* 117: 762–765, 1985.
3. **Anderson SM, Bowers CY, and Veldhuis JD.** Somatostatin-induced rebound growth hormone (GH) secretion unveils distinct mechanisms of action of GH-releasing hormone (GHRH) and GH-releasing peptide-2 (GHRP-2). *Ann Meeting Endocr Soc 84th San Francisco*, 2002.
4. **Anderson SM, Wideman L, Patrie JT, Weltman A, Bowers CY, and Veldhuis JD.** Estradiol supplementation selectively relieves growth hormone (GH)'s autonegative feedback on GH-releasing peptide-2 (GHRP-2)-stimulated GH secretion. *J Clin Endocrinol Metab* 86: 5904–5911, 2002.
5. **Bluet-Pajot MT, Epelbaum J, Gourdji D, Hammond C, and Kordon C.** Hypothalamic and hypophyseal regulation of growth hormone secretion. *Cell Mol Neurobiol* 18: 101–123, 1998.
6. **Chihara K, Minamitani N, Kaji N, Arimura A, and Fugita T.** Intraventricularly injected growth hormone stimulates somatostatin release into rat hypophyseal portal blood. *Endocrinology* 109: 2278–2281, 1981.
7. **Chomczynski P, Downs TR, and Frohman LA.** Feedback regulation of growth hormone (GH)-releasing hormone gene expression by GH in rat hypothalamus. *Mol Endocrinol* 2: 236–241, 1988.
8. **Clark RG, Carlsson LMS, Rafferty B, and Robinson ICAF.** The rebound release of growth hormone (GH) following somatostatin infusion in rats involves hypothalamic GH-releasing factor release. *J Endocrinol* 119: 397–404, 1988.
9. **Conway S, McCann SM, and Krulich L.** On the mechanism of growth hormone autofeedback regulation: possible role of somatostatin and growth hormone-releasing factor. *Endocrinology* 117: 2284–2292, 1985.
10. **Davis SL, Ohlson DL, Klindt J, and Anfinson MS.** Episodic growth hormone secretory patterns in sheep: relationship to gonadal steroid hormones. *Am J Physiol Endocrinol Metab Gastrointest Physiol* 233: E519–E523, 1977.
11. **De los Frailes MT, Cacicedo L, Fernandez G, Tolon RM, Jesus Lorenzo M, Aguado F, and Sanchez Franco F.** Role of locally produced growth hormone-releasing factor in somatostatin regulation by fetal rat brain cells in culture. *Neuroendocrinology* 55: 221–229, 1992.
12. **Epelbaum J, Moyse E, Tannenbaum GS, Kordon C, and Baudet A.** Combined autoradiographic and immunohistochemical evidence for an association of somatostatin binding sites with growth hormone-releasing factor containing nerve cell bodies in the rat arcuate neurons. *J Neuroendocrinol* 1: 109–115, 1989.
13. **Evans WS, Anderson SM, Hull LT, Azimi PP, Bowers CY, and Veldhuis JD.** Continuous 24-hour intravenous infusion of recombinant human growth hormone (GH)-releasing hormone-(1,44)-amide augments pulsatile, entropic, and daily rhythmic GH secretion in postmenopausal women equally in the estrogen-withdrawn and estrogen-supplemented states. *J Clin Endocrinol Metab* 86: 700–712, 2001.
14. **Farhy LS, Straume M, Johnson ML, Kovatchev BP, and Veldhuis JD.** A construct of interactive feedback control of the GH axis in the male. *Am J Physiol Regul Integr Comp Physiol* 281: R38–R51, 2001.
15. **Farhy LS, Straume M, Johnson ML, Kovatchev BP, and Veldhuis JD.** Unequal autonegative feedback by GH models the sexual dimorphism in GH secretory dynamics. *Am J Physiol Regul Integr Comp Physiol* 282: R753–R764, 2002.
16. **Fernandez Vazquez G, Cacicedo L, de los Frailes MT, Lorenzo MJ, Tolon R, and Sanchez Franco F.** Growth hormone-releasing factor regulation by somatostatin, growth hormone and insulin-like growth factor I in fetal rat hypothalamic-brain stem cell cocultures. *Neuroendocrinology* 58: 655–665, 1993.

17. **Friesen WO, Block GD, and Hocker CG.** Formal approaches to understanding biological oscillators. *Annu Rev Physiol* 55: 661–681, 1993.
18. **Frohman LA, Downs TR, and Chomczynski P.** Regulation of growth hormone secretion. *Front Neuroendocrinol* 13: 344–405, 1992.
19. **Gevers E, Pincus SM, Robinson ICAF, and Veldhuis JD.** Differential orderliness of the GH release process in castrate male and female rats. *Am J Physiol Regul Integr Comp Physiol* 274: R437–R444, 1998.
20. **Giustina A and Veldhuis JD.** Pathophysiology of the neuroregulation of GH secretion in experimental animals and the human. *Endocr Rev* 19: 717–797, 1998.
21. **Hartman ML, Faria AC, Vance ML, Johnson ML, Thorner KG, and Veldhuis JD.** Temporal structure of in vivo growth hormone secretory events in man. *Am J Physiol Endocrinol Metab* 260: E101–E110, 1991.
22. **Hartman ML, Veldhuis JD, Johnson ML, Lee MM, Alberti KG, Samojlik E, and Thorner MO.** Augmented growth hormone (GH) secretory burst frequency and amplitude mediate enhanced GH secretion during a two-day fast in normal men. *J Clin Endocrinol Metab* 74: 757–765, 1992.
23. **Holl RW, Hartman ML, Veldhuis JD, Taylor WM, and Thorner MO.** Thirty-second sampling of plasma growth hormone in man: correlation with sleep stages. *J Clin Endocrinol Metab* 72: 854–861, 1991.
24. **Horvath S, Palkovits M, Gorcs T, and Arimura A.** Electron microscopic immunocytochemical evidence for the existence of bidirectional synaptic connections between growth hormone-releasing hormone- and somatostatin-containing neurons in the hypothalamus of the rat. *Brain Res* 481: 8–15, 1989.
25. **Iranmanesh A, South S, Liem AY, Clemmons D, Thorner MO, Weltman A, and Veldhuis JD.** Unequal impact of age, percentage body fat, and serum testosterone concentrations on the somatotrophic, IGF-I, and IGF-binding protein responses to a three-day intravenous growth-hormone-releasing-hormone (GHRH) pulsatile infusion. *Eur J Endocrinol* 139: 59–71, 1998.
26. **Jaffe CA, DeMott FR, and Barkan AL.** Involvement of endogenous growth hormone (GH)-releasing hormone in the GH responses to physiological and pharmacological stimuli. *J Clin Invest* 97: 934–940, 1996.
27. **Katakami H, Arimura A, and Frohman LA.** Growth hormone (GH)-releasing factor stimulates hypothalamic somatostatin release: an inhibitory feedback effect on GH secretion. *Endocrinology* 118: 1872–1877, 1986.
28. **Lanneau C, Bluet-Pajot MT, Zizzari P, Csaba Z, Dournaud P, Helboe L, Hoyer D, Pellegrini E, Tannenbaum GS, Epelbaum J, and Gardette R.** Involvement of the Sst1 somatostatin receptor subtype in the intrahypothalamic neuronal network regulating growth hormone secretion: an in vitro and in vivo antisense study. *Endocrinology* 141: 967–979, 2000.
29. **Lanzi R and Tannenbaum GS.** Time-dependent reduction and potentiation of growth hormone (GH) responsiveness to GH-releasing factor induced by exogenous GH: role for somatostatin. *Endocrinology* 130: 1822–1928, 1992.
30. **Lumpkin MD, Samson WK, and McCann SM.** Effects of intraventricular growth hormone-releasing factor on growth hormone release: further evidence for ultrashort loop feedback. *Endocrinology* 116: 2070–2074, 1985.
31. **Magnan E, Cataldi M, Guillaume V, Conte-Devolx B, Graziani N, Figaroli JC, Thomas F, Chihara K, and Oliver C.** Acute changes in growth hormone-releasing hormone secretion after injection of BIM 23014, a long acting somatostatin analog, in rams. *Life Sci* 51: 831–838, 1992.
32. **Maiter DM, Gabriel SM, Koenig JI, Russell WE, and Martin JB.** Sexual differentiation of growth hormone feedback effects on hypothalamic growth hormone-releasing hormone and somatostatin. *Neuroendocrinology* 51: 174–180, 1990.
33. **Miki N, Ono M, Miyoshi H, Tsushima T, and Shizume K.** Hypothalamic growth hormone-releasing factor (GRF) participates in the negative feedback regulation of growth hormone secretion. *Life Sci* 44: 469–476, 1981.
34. **Mitsugi N, Arita J, and Kimura F.** Effects of intracerebroventricular administration of growth hormone-releasing factor and corticotropin-releasing factor on somatostatin secretion into rat hypophysial portal blood. *Neuroendocrinology* 51: 93–96, 1990.
35. **Muller EE, Locatelli V, and Cocchi D.** Neuroendocrine control of growth hormone secretion. *Physiol Rev* 79: 511–607, 1999.
36. **Oliver C, Mical RS, and Porter JC.** Hypothalamic-pituitary vasculature: evidence for retrograde blood flow in the pituitary stalk. *Endocrinology* 101: 598–604, 1977.
37. **Pellegrini E, Bluet-Pajot MT, Mounier F, Bennett P, Kordon C, and Epelbaum J.** Central administration of a growth hormone (GH) receptor mRNA antisense increases GH pulsatility and decreases hypothalamic somatostatin expression in rats. *J Neurosci* 16: 8140–8148, 1996.
38. **Pellegrini E, Carmignac DF, Bluet-Pajot MT, Mounier F, Bennett P, Epelbaum J, and Robinson ICAF.** Intrahypothalamic growth hormone feedback: from dwarfism to acromegaly in the rat. *Endocrinology* 138: 4543–4551, 1997.
39. **Pincus SM.** Approximate entropy as a measure of system complexity. *Proc Natl Acad Sci USA* 88: 2297–2301, 1991.
40. **Pincus SM.** Quantifying complexity and regularity of neurobiological systems. *Methods Neurosci* 28: 336–363, 1995.
41. **Pincus SM, Gevers E, Robinson ICAF, van den Berg G, Roelfsema F, Hartman ML, and Veldhuis JD.** Females secrete growth hormone with more process irregularity than males in both human and rat. *Am J Physiol Endocrinol Metab* 270: E107–E115, 1996.
42. **Plotsky PM and Vale W.** Patterns of growth hormone-releasing factor and somatostatin secretion into the hypophysial-portal circulation of the rat. *Science* 230: 461–463, 1985.
43. **Robinson ICAF.** The growth hormone secretory pattern: a response to neuroendocrine signals. *Acta Paediatr Scand Suppl* 372: 70–78, 1991.
44. **Roelfsema F, Biermasz NR, Veldman RG, Veldhuis JD, Frolich M, Stokvis-Brantsma WH, and Wit JM.** Growth hormone (GH) secretion in patients with an inactivating defect of the growth hormone releasing-hormone (GHRH)-receptor is pulsatile: evidence for a role of non-GHRH inputs into the generation of GH pulses. *J Clin Endocrinol Metab* 86: 2459–2464, 2000.
45. **Stachura ME and Tyler JM.** Growth hormone-releasing factor-44 specificity for components of somatotroph and lactotroph immediate release pool substructures. *Endocrinology* 120: 1719–1726, 1987.
46. **Stachura ME, Tyler JM, and Farmer PK.** Combined effects of human growth hormone (GH)-releasing factor-44 (GRF) and somatostatin (SRIF) on post-SRIF rebound release of GH and prolactin: a model for GRF-SRIF modulation of secretion. *Endocrinology* 123: 1476–1482, 1988.
47. **Takahashi T, Okimura Y, Yoshimura K, Shigeyoshi Y, Kaji H, Abe H, and Chihara K.** Regional distribution of growth hormone-releasing hormone (GHRH) receptor mRNA in the rat brain. *Endocrinology* 136: 4721–4724, 1995.
48. **Van den Berg G, Veldhuis JD, Frolich M, and Roelfsema F.** An amplitude-specific divergence in the pulsatile mode of GH secretion underlies the gender difference in mean GH concentrations in men and premenopausal women. *J Clin Endocrinol Metab* 81: 2460–2466, 1996.
49. **Veldhuis JD, Fletcher TP, Gatford KL, Egan AR, and Clarke IJ.** Hypophysial-portal somatostatin (SRIF) and jugular venous growth hormone secretion in conscious unrestrained ewe. *Neuroendocrinology* 75: 83–91, 2002.
50. **Veldhuis JD, Johnson ML, Veldhuis OL, Straume M, and Pincus SM.** Impact of pulsatility on the ensemble orderliness (approximate entropy) of neurohormone secretion. *Am J Physiol Regul Integr Comp Physiol* 281: R1975–R1985, 2001.
51. **Veldhuis JD, Roemmich JN, and Rogol AD.** Gender and sexual maturation-dependent contrasts in the neuroregulation of growth-hormone (GH) secretion in prepubertal and late adolescent males and females. *J Clin Endocrinol Metab* 85: 2385–2394, 2000.
52. **Veldhuis JD, Straume M, Iranmanesh A, Mulligan T, Jaffe C, Barkan A, Johnson ML, and Pincus S.** Secretory process regularity monitors neuroendocrine feedback and feedforward signaling strength in humans. *Am J Physiol Regul Integr Comp Physiol* 280: R721–R729, 2001.