A construct of interactive feedback control of the GH axis in the male

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1Department of Internal Medicine, Division of Endocrinology and Metabolism, 2Departments of Psychiatric Medicine and Health Evaluation Sciences, 3Department of Pharmacology, The University of Virginia Health System, 4Center for Biomathematical Technology, and 5National Science Foundation Center for Biological Timing, University of Virginia, Charlottesville, Virginia 22908

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Farhy, Leon S., Martin Straume, Michael L. Johnson, Boris Kovatchev, and Johannes D. Veldhuis. A construct of interactive feedback control of the GH axis in the male. Am J Physiol Regulatory Integrative Comp Physiol 281: R38–R51, 2001.—Growth hormone (GH) secretion is controlled by GH-releasing hormone (GHRH), the GH releasing-inhibiting hormone somatostatin (SRIF), and autofeedback connections. The ensemble network produces sexually dimorphic patterns of GH secretion. In an effort to formalize this system, we implemented a deterministically based autonomous feedback-driven construct of five principal dose-responsive regulatory interactions: GHRH drive of GH pituitary release, competitive inhibition of GH release by SRIF, GH autofeedback via SRIF with a time delay, delayed GH autonegative feedback on GHRH, and SRIF inhibition of GHRH secretion. This formulation engenders a malelike pattern of successive GH volleys due jointly to positive time-delayed feedback of GH on SRIF and negative feedback of SRIF on GH and GHRH. The multipeak volley is explicated as arising from a reciprocal interaction between GH and GHRH during periods of low SRIF secretion. The applicability of this formalism to neuroendocrine control is explored by initial parameter sensitivity analysis and is illustrated for selected feedback-dependent experimental paradigms. The present construct is not overparameterized and does not require an ad hoc pulse generator to achieve pulsatile GH output. Further evolution of interactive constructs could aid in exploring more complex feedback postulates that confer the vivid sexual dimorphism of female GH profiles.

growth hormone; somatostatin; growth hormone-releasing hormone; hypothalamus; mathematical model

EXTENSIVE EVIDENCE supports the notion that the physiological control of growth hormone (GH) secretion is governed at least by a hypothalamic releasing hormone, GH-releasing hormone (GHRH), and a hypothalamic GH release-inhibiting hormone, somatostatin (SRIF) (17, 39, 47, 48, 51, 54). These pivotal neuroregulatory peptides are secreted by specialized and interconnected mediobasal and periventricular neurons into hypophyseal portal blood and then transported to the anterior pituitary gland. GHRH drives somatotrope cell biosynthesis and release of GH, whereas SRIF antagonizes GH secretion per se. Systemic GH exerts negative feedback on its own secretion primarily via hypothalamic actions, which enhance the secretion of SRIF and limit the release of GHRH. These fundamental connections are assumed in ensemble to endow pulsatile GH secretory profiles with unique dynamics. For example, the GH secretion profile in the adult male rat is remarkable for its typically multipeak release episodes, which recur at ∼3.3-h intervals separated by undetectable interpulse or trough periods (49).

GH-release patterns are markedly pulsatile in all species studied (9, 14, 18, 21, 23, 32, 39, 45) and typically sexually dimorphic (18, 20, 21, 38). The particular pattern of GH output is critical to greater body growth in the male and the regulated sex-specific expression of selected genes in target cells of GH action (21). For example, the IGF-I gene is induced differentially in muscle and liver by a pulsatile (malelike) and continuous (femalelike) GH signal. Experiments in the prepubertal rat using surgical and pharmacological gonadal ablation unmask the capability of this axis to generate a full spectrum of male-to-female (pulsatile to nearly continuous) modes of GH secretion (20). Other studies in the adult of this species have disclosed rapid modulation by exogenous sex steroids of GH secretory patterns (35, 36). Such observations strongly suggest plasticity of the interactive neuroendocrine mechanisms that generate rhythmic patterns of GH secretion. However, intuitive reconstruction of time-delayed nonlinear interactive feedback changes is challenging. Accordingly, we here consider simplified biomathematical modeling of the ensemble interactions as a complementary tool to unravel the complex and likely multivalent nature of adaptive neuroregulatory mechanisms that control GH output.

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An earlier biomathematical network by Chen et al. (7) to embody rhythmic GH drive in the rat was constrained by its high parametric (>70) complexity, which is a serious obstacle to exploring the behavior of the system facilely. A second model by Wagner et al. (53) achieves simplicity by incorporating an autonomous GHRH pulse generator, which imposes two consecutive pulses within each secretory episode. These preliminary formulations of GH dynamics thereby pose the critical physiological issue: whether relevant feedback connectivity alone can initiate and sustain such recurrent volleys of pulsatile GH secretion. In addition, neither construct has been shown to achieve the more complex dynamics of rebound GH secretion, GH autofeedback on GHRH, graded features of sexual dimorphism of GH secretion (above), and/or aging-related alterations in GH pattern reproducibility (21). A third model by Brown et al. (4) explores the kinetics of the pituitary GH secretory response to hypothalamic GHRH stimulation, while allowing for the suppressive effect of concurrent SRIF concentrations, but does not interlink ongoing GH-GHRH-SRIF feedback as a time-evolving dynamic.

In an effort to extend the foregoing important insights, the present work formulates the basic GH secretion pattern in the male rat in a simplified construction arising from the primary consensus interactions in the system among GH, GHRH, and SRIF (15, 18, 21, 23, 26, 27, 34, 40, 45, 47–50, 53). We show that this deterministic representation can evolve dynamic feedback and feed-forward-sensitive GH output over time while exhibiting stochastic features arising solely from the nodal interactions.

**Fig. 1.** Schema of principal regulatory connections within the growth hormone (GH) feedback network. Downregulatory interactions [including elimination (elim.) processes] are denoted with lines ending with solid circles, whereas upregulatory interactions correspond to "T" endings. The up- and downregulatory dose-dependent interactions are numbered consecutively from 1 to 5 as described in the text. See METHODS for details. P, pituitary gland; GHRH, GH-releasing hormone; SRIF, somatostatin.

**METHODS**

**Network Connectivity Within the GH Axis**

The basic within-axis regulatory features, which are postulated in ensemble to drive the episodic release of GH, are presented schematically in Fig. 1.

The network consists of five principal feed-forward and feedback interactions as well as an expected elimination and release process for each of GH, GHRH, and SRIF. The release processes are affected by the interactions, whereas the elimination processes are assumed to remain largely stable. Briefly, the core regulatory interactions that impact GH secretion are as follows. 1) GHRH directly and dose responsively drives pituitary GH release in vitro and in vivo (21, 27). Biochemical studies have shown that somatotrope GHRH receptors mediate intracellular cAMP generation, Ca2+-dependent GH gene transcription, and the exocytosis of GH-containing secretory granules (34). 2) Higher concentrations of SRIF inhibit GHRH-driven GH secretion via a family of SRIF receptors, which variously mediate inhibition of GHRH-stimulated adenyl cyclase via Gi, Ca2+-channel conductance, somatotrope cell-membrane hyperpolarization, and nuclear phosphatase activation (11, 27). 3) Autofeedback by GH via brain GH receptors (37, 41) increases the release of hypothalamic SRIF with a delay of 40–60 min in the adult male rat (8), probably acting via SRIF-receptor subtype 2 receptors to restrain GH secretion (56). 4) SRIF negatively regulates the release of GHRH from the hypothalamus into the portal blood. This interaction is evident both in vitro and in vivo experiments (21) and is structurally defined by synaptic associations of SRIF neurons on a subpopulation of GHRH-containing and SRIF receptor-expressing arcuate nucleus neurons (16, 49). 5) GH autonegative feedback suppresses GHRH gene expression and GHRH secretion (3, 10, 21, 28, 34, 40, 44). We hypothesize that the inhibiting effect on hypothalamic GHRH release is time delayed to account for the collective decay of neuronal GHRH secretion, capillary clearance of GHRH, dissociation of prior GHRH bound to GH receptors, and extinction of the GH secretory response.

Interactions 1 and 2 are assumed to occur nearly simultaneously as observed in vitro; i.e., GH release is possible only if GHRH concentrations are high and SRIF levels are relatively low. Analogously, in vivo experiments show an amplified GH secretory response to GHRH injections during ongoing GH secretory bursts (when SRIF is low) and, conversely, impaired GH release when GHRH is infused during a GH trough period corresponding to higher SRIF levels (48).

Interactions 4 and 5 are also assumed to be concurrent, so that GHRH release occurs only when both previous GH and SRIF levels are low. In this regard, Plofsky and Vale (39) observed that GH and GHRH are released concomitantly with SRIF withdrawal in the anesthetized adult male rat. On the basis of more intensive blood sampling in the conscious ovariectomized sheep (19), GHRH release also consistently (~70%) precedes GH secretory bursts.

The foregoing basic interactions are well established by multiple independent investigations in the rat. We thus next ask whether this core of relationships is sufficient to explain the typical pattern of spontaneous GH release in the male rat.

**Core Equations**

We model each of GH, SRIF, and GHRH as released continuously at feedback-specified rates. Each peptide undergoes time-invariant and hormone-specific exponential clear-
ance. However, for GH and GHRH (but not SRIF), the rate of release is controlled by feed-forward and/or feedback inputs from the other two peptides. In the case of SRIF, feedback is here construed as only via GH, albeit indirect evidence exists for GHRH and/or SRIF (autofeedback also under some conditions (21)).

To describe the rate of change of the GH concentration in the circulation, we assume that the SRIF concentration exert a negative effect on the release of GH, whereas the GHRH level stimulates GH secretion. Thus the rate of change of GH concentration with respect to time is given by

\[ \frac{d[GH]}{dt} = GH' = -k_1 GH + k_2 [F_1(GHRH)F_2^*(SRIF)] \tag{1} \]

where \( t \) is the time, \( k_1 \) and \( k_2 \) are the rate constants of elimination and release, respectively, and \( F_1^* \) and \( F_2^* \) are the corresponding up- and downregulatory driving functions, respectively, for which we use corresponding Hill functions (7)

\[ F_1^*(GHRH) = \frac{(GHRH/t_1)^{n_1}}{(GHRH/t_1)^{n_1} + 1} \]

\[ F_2^*(SRIF) = \left[ 1 - \frac{(SRIF/t_2)^{n_2}}{(SRIF/t_2)^{n_2} + 1} \right] \]

where \( t_1 \) and \( t_2 \) and \( n_1 \) and \( n_2 \) are thresholds and Hill coefficients, respectively, for the two regulatory functions acting on GHRH and SRIF (for motivational details, see Chen et al. (7)). At relatively high Hill coefficients, the thresholds are the concentrations, near which the corresponding effects emerge. The multiplicative form is used to ensure that GH release can occur only when SRIF levels are low and when GHRH is capable of achieving stimulation.

The rate of change of the SRIF concentration is assumed to be positively affected, after a certain time delay constant (\( D \)), by the systemic GH concentration. We supposed that SRIF concentrations remain above a nonzero basal level, as documented by frequent hypothalamo-hypophyseal portal blood sampling (19). Thus we can describe the time rate of change of SRIF concentration into the portal blood by

\[ \frac{d[SRIF]}{dt} = SRIF' = -k_2 SRIF + k_3 [F_1^*[GH(t - D)]] \tag{2} \]

where \( k_2 \) is the rate constant of disappearance and \( k_3 \) is the rate constant of release. The upregulatory function is approximated by a Michaelis-Menten-like competitive equation (7, 29)

\[ F_1^*[GH(t - D)] = \left[ \frac{S_{min} - 1}{1 + GH(t - D)/t_3} + 1 \right] \]

where \( S_{min} \) is the minimal attainable magnitude of the regulatory function and \( t_3 \) is the Michaelis-Menten constant. Thus the basal SRIF level is specified by \( S_{min} \).

The rate of change of the GHRH concentration in the portal blood is based on the assumption that both GH and SRIF suppress GHRH (above) and that GH suppression of GHRH is time delayed (3, 10, 21, 28, 34, 40, 44). This twofold suppression is incorporated by dual downregulatory functions, which are combined so that release of GHRH occurs only when both GH [before a time delay constant (\( T \))] and SRIF tend to be low values. Therefore

\[ \frac{d[GHRH]}{dt} = GHRH' \]

\[ = -k_0 GHRH + k_3 [F_4^*[GH(t - T)F_5^*(SRIF)]] \tag{3} \]

where \( k_3 \) and \( k_{r,3} \) are the rate constants of GHRH clearance and release, respectively. The corresponding dose-response interface functions are

\[ F_4^*(GH) = \frac{1}{[GH(t - T)/t_4]^{n_4} + 1} \]

\[ F_5^*(SRIF) = \frac{1}{(SRIF/t_5)^{n_5} + 1} \]

with thresholds \( t_4 \) and \( t_5 \) and Hill coefficients \( n_4 \) and \( n_5 \).

Combining Eqs. 1–3 yields the following core system of coupled nonlinear ordinary differential equations

\[ GH' = -k_1 GH + k_2 \left[ \frac{(GHRH/t_1)^{n_1}}{(GHRH/t_1)^{n_1} + 1} \right] \]

\[ SRIF' = -k_2 SRIF + k_3 \left[ \frac{S_{min} - 1}{1 + GH(t - D)/t_3} + 1 \right] \]

\[ GHRH' = -k_0 GHRH + k_3 \left[ \frac{1}{[GH(t - T)/t_4]^{n_4} + 1} \right] \]

\[ \left[ \frac{1}{(SRIF/t_5)^{n_5} + 1} \right] \]

**Definition of Parameters in the Reference System**

The clearance constant \( k_1 \) corresponds to the half-life of GH, which in the adult male rat approximates 15.5 min, as measured directly by Chapman et al. (6). Thus \( k_1 \) is provisionally fixed to 2.7/h in our system for normal male physiology.

The value of \( k_{r,1}/k_1 \) is the maximal attainable GH concentration in the portal blood during periods of low SRIF and high GHRH levels (including response to external GHRH challenge). Here, we arbitrarily posit that in the adult male rat, circulating GH concentrations rarely exceed 2,140 ng/ml. Thus \( k_{r,1} = k_1 \times 2,140 \text{ ng/ml} = 5,775 \text{ ng/ml} \cdot \text{h}^{-1} \). Although this nominal value likely exhibits interindividual variation among animals. For example, occasional experiments (48) report rare serum GH concentrations as high as 3,000 ng/ml.

The delay between the increase in GH in systemic blood and the rise in SRIF is experimentally estimated to be in the range of 40–60 min (8). Here, we accept a nominal delay of 60 min, which defines the value of \( D \) as 1 h. This parameter could also vary in principle both within and among animals. Indeed, we speculate that such variations could account for the occurrence of sustained succession of GH peaks within a major secretory episode, as is evident at times in the human (22).

The half-lives of SRIF \( k_2 \) and GHRH \( k_3 \) are chosen to correspond to the “effective” half-lives of the two peptides; i.e., their availability to target cells to exert feedback effects. The literature contains no definitive data to determine these values a priori. Here, we estimate functional SRIF and GHRH half-lives that allow for at least two consecutive peaks within any given major secretory episode. According to this assessment, we have estimated \( k_2 = 8 \text{ h} \) and \( k_3 = 5 \text{ h} \). The latter conforms to a periodicity of major secretory episodes of \( \sim 3.3 \text{ h} \). Both values necessarily approach or exceed the whole body half-lives of these peptides, namely \( \sim 2–7 \text{ min} \) (21).

The rate constants \( k_{r,2} \) and \( k_{r,3} \) correspond to the maximally observed values for SRIF and GHRH, respectively. For SRIF, we assumed a maximal effect of \( \sim 1,050 \text{ pg/ml} \) (8); so that \( k_{r,2} = 5,250 \text{ pg/ml} \cdot \text{h}^{-1} \). There are insufficient data to assign the corresponding value for GHRH. Empirically, we estimated \( k_{r,3} = 76,800 \text{ pg/m}l \cdot \text{h}^{-1} \), wherein GH rebound after a 4-h continuous SRIF infusion emulates the experimental data of Clark et al. (11).
The basal SRIF concentration is assumed to be \( 22 \text{ pg/ml} \), as suggested by some experimental data (39). This nominal value would determine \( S_{\text{min}} \) from the equation
\[
k_{r,2} S_{\text{min}} = 22 \text{ pg/ml}.
\]

Under the foregoing provisional parameter set, the sole parameters requiring estimation are those of the regulatory (dose responsive) Hill functions. By definition, the thresholds \( t_1 \) and \( t_4 \) should be smaller than the maximally attainable concentrations of GH and GHRH during a typical GH burst to serve as physiological parameters. The thresholds \( t_2 \) and \( t_5 \) designate disinhibition of SRIF and are provisionally set close to the basal SRIF level. The values of the exponents in the Hill functions would be higher when the regulatory functions allow more rapid burstlike GH secretory activity.

The Michaelis-Menten constant \( t_3 \) was empirically estimated to allow for a dose-related SRIF response to intracerebroventricular (icv) infusion of homologous GH (8). The complete set of parameters is shown in Table 1.

The numerical integration of the nonlinear differential equations was performed using the subroutines in Mathematica, which implement Runge-Kutta method 4–5 for non-stiff equations with an adaptive procedure for determining the size of the step (55).

### RESULTS

**Basic Model Output**

The model profiles for the reference (unperturbed) GH, SRIF, and GHRH concentration vs. time plots are presented below. To be sure that the dynamic properties are stable in time, we followed the solution for 91 h (Fig. 2).

Next, we explored specific mechanisms that might drive the particular rhythmicity of the GH concentration profile in the male rat. To this end, we examined the dynamic reactions inherent in this feedback construct. This investigation of the parameter sensitivity of system behavior is illustrated schematically in Fig. 3A.

The plot is divided into two regions of relative SRIF dominance or withdrawal, which we have operationally designated the restrictive zone and the permissive zone. In the restrictive zone, SRIF concentrations exceed a certain level above which SRIF effectively inhibits both GH and GHRH release and thus acts restrictively. Any small GHRH increase in the restrictive zone would not have the necessary amplitude to evoke GH secretion, which is suppressed by high SRIF. Conversely, when decaying SRIF levels become permissive, the (first) pulse of GHRH is released into portal blood and GHRH-driven secretion of GH is initiated (to drive GH, the GHRH level approaches its action threshold \( t_1 \)). Approximately 7 min (\( T \)) after GH approaches its feedback threshold \( t_4 \), suppression of GHRH release commences, allowing the GHRH concentration to decline to undetectable values due to elimination. The rise in GH output continues as GHRH falls below its stimulatory threshold. Then, GH secretion wanes, and GH concentrations decay due to elimination and to low GHRH stimulation. Diminished GH levels will then allow gradual resumption of the release of GHRH. This

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### Table 1. Values of the parameters used in the reference model

<table>
<thead>
<tr>
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<th>Rate Constants</th>
<th>Release Constants</th>
<th>Thresholds</th>
<th>Hill Coefficients</th>
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</thead>
<tbody>
<tr>
<td>GH</td>
<td>( k_1 = 2.7/h )</td>
<td>( k_{r,1} = 5,575 \text{ng·ml}^{-1}·h^{-1} )</td>
<td>( t_4 = 47 \text{ng/ml} )</td>
<td>( n_4 = 3.5 )</td>
</tr>
<tr>
<td>SRIF</td>
<td>( k_2 = 5/h )</td>
<td>( k_{r,2} = 5,250 \text{pg·ml}^{-1}·h^{-1} )</td>
<td>( t_2 = 35 \text{pg/ml} )</td>
<td>( n_2 = 3.5 )</td>
</tr>
<tr>
<td>GHRH</td>
<td>( k_3 = 8/h )</td>
<td>( k_{r,3} = 76,800 \text{pg·ml}^{-1}·h^{-1} )</td>
<td>( t_5 = 22 \text{pg/ml} )</td>
<td>( n_5 = 3.5 )</td>
</tr>
</tbody>
</table>

Delay constants: \( D = 1 \text{ h} \), \( T = 6.912 \text{ min} \). Equation for minimal attainable magnitude (\( S_{\text{min}} \)): \( k_{r,2} S_{\text{min}} = 110 \text{ pg·ml}^{-1}·h^{-1} \). Michaelis-Menten constant: \( t_3 = 1,166.7 \text{ ng/ml} \). GH, growth hormone; SRIF, somatostatin; GHRH, GH-releasing hormone.

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![Fig. 2. Illustrative output for model-driven SRIF, GHRH, and GH profiles. The upper curve corresponds to model-specified GH release, and that at the bottom corresponds to SRIF release. The solution of the core coupled ordinary differential equations was followed for 91 h (region 75–91 plotted here) to establish stable properties.](http://ajpregu.physiology.org/)

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Fig. 3. A: schematic representation of model behavior in the male rat. The plot is divided into different zones corresponding to different states of the system. Two rectangles are shown to illustrate the delayed feedback actions of GH on GHRH (the smaller rectangle) and SRIF. The left sides of both rectangles cross the GH profile at points, in which the corresponding feedback effect was initiated. The right sides cross the GHRH and SRIF profiles at points in which the corresponding feedback action can be observed. B: simulation of the impact of SRIF removal on system behavior. C: illustrative explanation of the terms “intravolley” (the distance between two peaks within a volley; denoted with a 1) and “intervolley” (the distance between the first 2 peaks in 2 recurrent volleys; denoted with 2 in the picture) intervals. The evident duration of a complete volley is denoted with 3.
reciprocal interaction between GHRH and GH produces the first peak in the secretory volley. The second GH peak evolves analogously and typically at a lower amplitude due to the elevated SRIF concentrations. (The latter is not obligatory, based on the system parameter choice, and might vary among species.) More GH peaks would appear, but GH autofeedback activated by the first peak evokes SRIF secretion with a time delay (here, 60 min). The system enters the restrictive zone, wherein the rising SRIF level suppresses the release of GH and GHRH, in which concentrations fall unidirectionally due to metabolic elimination. The SRIF profile thus accompanies the GH profile but with the specified time delay. Conditional on the decay of GH and with a 60-min delay, SRIF concentrations fall. When the SRIF level becomes permissive (and remains permissive for >60 min), a new volley of GH secretion emerges.

To demonstrate more explicitly the GH-GHRH interactions responsible for the multiple peaks during a volley, we have simulated the removal of SRIF [by replacing the term SRIF(t) in the first and third model equations by 0]. Delay of GH's autofeedback effect on GHRH provides oscillations with a period of ~1 h. This high frequency would emulate the female pattern. The model output is presented on Fig. 3B. Hartman et al. (22) suggested the terms intervolley (time interval between the first events in successive multipeaked episodes of GH secretion) and intravolley (time between consecutive peaks within a volley) intervals (see Fig. 3C).

The foregoing construct imputes rather different feedback underpinnings for these two intervals. First, the intravolley interval is shorter than the intervolley delay. The shorter intravolley time is explained by the combination of thresholds and/or elimination rates for GH and GHRH and the time delay in the GH autofeedback on GHRH. Specifically, the second peak within a volley emerges before the complete decay of GH, as disinhibition of GHRH secretion abates. The larger intervolley interval arises because of the combination of time delays for peripheral GH's feedback action on SRIF (which specifies the actual volley duration) and the GH elimination rate in the systemic circulation. The latter is important in allowing high systemic GH levels to maintain high SRIF secretion, thus enforcing restrictive effects between volleys. The restrictive zone emerges from properties of the regulatory function \( F_3^{-1} \), which allows for even small GH concentrations to maintain heightened SRIF release. Thus the interval between successive peaks within any given GH volley is set by the autofeedback kinetics of GH and GHRH (during SRIF withdrawal). The evident duration of any given complete volley (see Fig. 3C) is due to the time delay between the first GH peak in the volley and effective GH feed forward on SRIF. And, the intervolley interval is set by the sum of the particular prior volley length and the decay time of systemic GH to allow adequate relief of the prior SRIF elevation. This interpretation also recognizes the considerably shorter off times for GHRH and SRIF actions than that for the systemic elimination of GH.

**Model reactivity to defined interventions.** To examine dynamic reconstruction of selected physiological observations, we have explored model reactivity to published experimental interventions. Note that the model is intended to recapitulate only appropriate outputs but not analytically fit data.

**GH rebound following short-term SRIF infusion and subsequent withdrawal.**

**CONTINUOUS SRIF INFUSION.** The effects of short-term intravenous infusion and subsequent withdrawal of SRIF were simulated by adding an additional term [the exogenous SRIF infusion \( (S_{\text{inf}}) \)] to the argument of \( F_2 \). The first equation is then written in the form

\[
GH' = -k_1 GH + k_{r,1} \frac{(GHRH(t_1))^{n_1}}{(GHRH(t_1))^{n_1} + 1} \left( \frac{1}{([SRIF+S_{\text{inf}}]/t_2)^{n_2} + 1} \right)
\]

The SRIF infusion was chosen to be continuous from 0400 to 0800 (Fig. 4). This way of introducing the

![Fig. 4. Simulation of rebound GH release after cessation of a brief (4 h) SRIF infusion. The solid bar at the top denotes the period of SRIF infusion.](http://ajpregu.physiology.org/.../S43FEEDBACKCONTROLOFMALEGHAXIS.png)
perturbation is appropriate if peripheral infusion of SRIF only affects the release of GH driven by GHRH, i.e., it has no impact on GHRH or SRIF release into portal blood. The model output recapitulates the rebound release of GH reported in in vivo experiments (e.g., Fig. 1 in Ref. 11). In this construction, GH rebound is due to hypothalamic release of GHRH, as inferred by Clark et al. (11). During infusion of SRIF, the GHRH-driven release of GH is inhibited and GH decays due to elimination. As GH concentrations fall, hypothalamic release of SRIF also decreases. When hypothalamic SRIF falls sufficiently, GHRH release reemerges. However, exogenous SRIF blocks GH secretion and hence obviates its restraint of GHRH secretion, thereby promoting a rise in portal blood GHRH. After SRIF withdrawal, the high concentration of GHRH causes a large reboundlike release of GH, as observed.

**INTERMITTENT SRIF INFUSION.** An alternative simulation of SRIF actions entails its intermittent delivery: e.g., a 12-h SRIF infusion interrupted for 0.5 h every 3 h. This type of experiment was performed twice with the following differences. First, we imposed a continuous intravenous infusion of human GH during the third SRIF infusion period (see Fig. 5A). Second, we simulated the infusion of different amounts of GHRH antisera 8 h after the onset of SRIF infusion (see Fig. 5, B and C). GH infusion was simulated by adding an additional term (a constant during the infusion period) to the right-hand side of the first equation of the core system. To simulate partial or complete GHRH withdrawal due to variable immunization with GHRH antisera, the elimination rate of GHRH was increased 15- and 50-fold, starting 7.5 h after the onset of SRIF infusion. All output curves agree well with the published experiments of Clark et al. (11).

We note in Fig. 5 an initially unexpected secondary increase in GHRH release after the onset of the SRIF infusion. As noted above, in fact, this arises from the suppressive effect of exogenous SRIF on GH secretion, the decline of which disinhibits GHRH release while levels of hypothalamic SRIF are also low and permissive of GHRH release. Thus we infer that exogenous SRIF annihilates the second peak in the ongoing GH burst by suppressing GH but not GHRH release.

**GHRH injections during GH peak and trough periods.** We next tested the response of the model to GHRH injections during GH peak and trough periods in two related experiments. The first simulated two identical and consecutive GHRH injections at 1303 and 1503. The time of the first injection was chosen to match peak GH secretion, and the second injection was during a trough period. The response of the system is shown in Fig. 6A.

Model output corresponds to the observations in Refs. 27 and 48: i.e., large GH secretory response occurs during the GH peak and attenuated responses during the trough period. The large bursts are due to low permissive SRIF levels during the first infusion and the merely undetectable response to the second injection, to high, restrictive SRIF levels during the trough period. The next experiment tests this concept, and to the initially simulated effect of two identical GHRH injections made at 1303 and 1503, we imposed putative immunoneutralization of SRIF (Fig. 6B) by decreasing the term $SRIF(t)$ in the first model equation 30-fold starting at 0930. Here, we assume that only a small part of the SRIF antiserum crosses the brain-blood barrier to exert its effect on hypothalamic SRIF or GHRH neurons. Therefore, we decrease the term $SRIF(t)$ in the third model equation only fourfold starting at 0930. Output of the feedback model agrees well with experimental observations (48).
Human GH injection and the effect of SRIF antagonism. The effect of introducing an exogenous human GH injection at 0300. The bolus was modeled by an additional “GH secretion” term in the arguments of the regulatory functions F3 and F4. We implemented a high dose of exogenous human GH with slower elimination in the rat circulation (compared with endogenous GH), as observed in the published experiments. The model response is shown on Fig. 7.

Immunization with SRIF antiserum was modeled similarly to that above; i.e., decreasing the term SRIF(t) in the right-hand side of the first and third equations by 15- and 2-fold, respectively, starting at 0500 (accounting, as above, for a partial effect of SRIF antibodies on hypothalamic SRIF). The model output in this experiment is shown in Fig. 8A. Varying antibody efficiency further modifies the resultant GH profile, as shown in the rest of Fig. 8. Separately, we show the predicted effect of constant hypothalamic SRIF secretion, which depresses the GHRH release independently of injected antibodies.

Simulated infusion of GHRH antibodies. The effect of introducing dose-varying antiserum to GHRH is shown in Fig. 9. GHRH withdrawal was simulated, as described in INTERMITTENT SRIF INFUSION, by increasing the elimination rate of GHRH by 5- and 150-fold starting at 0900. The model-predicted GH profile agrees well with results of the reported protocols (54).

Stimulation of SRIF by an exogenous GH bolus. We have simulated the effect of icv infusion of homologous GH on the profile of SRIF. This experiment was reported by Chihara et al. (8), revealing a dose-related response to exogenous GH bolus in SRIF concentrations in portal blood with a peak value 20–80 min thereafter. The original experiment was performed on male rats anesthetized with urethane, which can itself alter the systemic GH profile (39). Therefore, the present aim was only to evaluate whether a GH bolus evokes a dose-related increase in SRIF release (see Fig. 10).
We performed two simulations of different (rat) GH icv injections introduced at 0430. The GH bolus was modeled as above (see Human GH injection and the effect of SRIF antagonism). To match the experiment of Chihara et al. (8), the second rat GH injection was increased fivefold. This elicited a peak that was \(1.3\) times greater and an area under the SRIF curve that was four- to sixfold larger in general assessment, with the onset of SRIF increase 60 min after the GH injection.

Model Reactivity to Specific Parameter Changes: Partial Parameter Sensitivity Analysis

Model sensitivity to changes in feedback delays. First, we examine model reactivity to changes in delays imposed on GH feedback on GHRH and SRIF. This choice is motivated by the assumption that delays are subject to deviations from their mean value among animals and to a lesser extent within a single animal. We have performed two simulations concerning the delay \(D\). In the first, we assume that \(D = 2\) h, and in the second, \(D = 0.5\) h. The longer delay causes a third peak to emerge within a single volley (see Fig. 11), whereas the shorter delay results in suppression of the second peak due to premature rise in SRIF levels. These patterns thereby emulate the rat (bipartite GH peak) and human [multiple GH excursions within a volley; see Hartman et al. (22)].

An increase in the second delay value \(T\) acts similarly to the decrease of \(D\) (the second peak within a volley disappears). However, the intervolley interval remains the same, whereas the decrease of \(D\) leads to an intervolley interval of \(~2\) h. Decreasing the delay \(T\) toward zero still preserves some of the key features of the male pattern. The intravolley interval decreases, which evokes a third peak within a single volley (see Fig. 12).

However, the peaks are not so pronounced except at very high Hill coefficients \(n_1\) and \(n_5\) (effective size of 40; see Fig. 13 and Table 2 for the particular parameter choice). The latter would correspond to regulatory processes with substantial cooperativity, which can induce thresholdlike sharp transitions in dose-response relationships, as observed in some simpler biological systems (see, for example, Refs. 17, 30, 33, 52). Thus \(T\) approaching zero is less realistic, we infer.

Partial parameter sensitivity analysis. By testing parameter predictions individually (in comparison with their initial values; Table 1), we could establish nominal operating intervals, within which the male rat model output is preserved qualitatively as recurrent

Fig. 8. A: simulation of the effect of introducing SRIF antibodies at 0500 [2 h after the human GH bolus injection (see Fig. 7)]. A: the function that augments the elimination constant of peripheral SRIF to simulate the introduction of antibodies. B: endogenous GH concentration profile obtained by unequal amounts of SRIF antiserum imposed to alter the feedback properties of exogenous human GH.

Fig. 9. Simulation of partial and complete GHRH withdrawal. When the reduction in GHRH is small (top), GHRH concentrations still approach the GHRH action threshold and elicit GH bursts. The interval between attenuated bursts is somewhat smaller. When the reduction in GHRH is large (bottom), GHRH concentrations far below the GHRH action threshold and GH bursts are not elicited.
Fig. 10. Simulated effects of a single intracerebroventricular injection of rat GH. The 2 plots correspond to introduction of different amounts of GH. The light line corresponds to the larger injection. The arrow shows the time of injection.

Fig. 11. Simulated effect of increasing 2-fold the delay time D. The permissive zone is larger, and 3 GH peaks are elicited within a single volley. The intermediate peak is larger because the corresponding SRIF levels are lower. The last peak is significantly attenuated due to rise in SRIF secretion.

Fig. 12. Simulated effect of decreasing 4-fold the delay time T. The intravolley interval is smaller, and 3 GH peaks emerge within a single volley.
multiphasic volleys. The results are presented in Table 3.

**DISCUSSION**

A premise of the present work is that unraveling the complex but regulated physiology of the dynamic GH axis requires a detailed understanding of the jointly interactive neuroendocrine mechanisms that underlie its changing secretion patterns. This expectation is challenged by the combined nonlinear, dose-responsive, time-delayed, and interconnected nature of the SRIF-GHRH-GH feed-forward and feedback ensemble and its evident automaticity. As an initial formalism to embody some of these ensemble relationships, we have formulated linkages within the GH axis based solely on the primary GH/GHRH/SRIF interactions that are well established experimentally by multiple independent laboratories (15, 21, 34, 44, 47). Albeit simplified, this deterministic structure generates recurring feed-forward- and feedback-dependent volleys of autonomous GH secretion with physiologically entrained intravolley GH pulses in the absence of marked overparametrization (7) or an externally driven GHRH pulse generator (53). Thereby, we exemplify that core feedback relationships with nominal physiological time delays and dose-response properties can emulate the expected complex ultradian rhythmicity of GH release in the male system.

Analysis of feedback parameter sensitivity unmasked the ability of the adult male rat GH “pulse within pulse” (or volleylike) secretory pattern to evolve approximately every 3.3 h due to a critical interplay between time-delayed GH autofeedback on GHRH, as modulated by concurrent SRIF levels. This negative feedback action of GH on GHRH gene expression has been documented via central nervous system GH receptor-dependent restraint of GH output, GH receptor expression on GHRH neurons, and neurophysiological data both in wild-type and in transgenic rodents (3, 10, 21, 28, 34, 40, 44). In particular, low SRIF output elevates GHRH secretion, which, in turn, drives the first peak of GH release in a multiphasic volley. By way of delayed autofeedback, GH from the first pulse in the volley exerts negative feedback on GHRH release, thereby generating a short intravolley interval. In particular, a slightly delayed autonegative feedback of GH on GHRH (even combined with relatively shallow dose-response interfaces, Hill coefficients of 3.5; see METHODS) can create more rapidly recurrent GH peaks within a larger secretory volley (for details, see Fig. 3A and the corresponding text).

Escape from GH autofeedback on GHRH neurons is achieved by sufficient decay of GH concentrations within a volley to relieve repression of GHRH (but not GH’s feed forward on SRIF). Conversely, a full volley of GH secretion is terminated when its GH peaks trigger the time-delayed output of SRIF. If the pituitary gland can secrete directly to the brain (2), and on the basis of the icv GH infusion data of Chihara et al. (8), maximal GH autofeedback-induced SRIF-dependent termination of a GH volley would thus occur within 40–60 min as predicted here and observed in vivo. Thus the apparent duration of a spontaneous GH volley (as distinct from the intervolley

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rate Constants</th>
<th>Release Constants</th>
<th>Thresholds</th>
<th>Hill Coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH</td>
<td>$k_1 = 2.5/h$</td>
<td>$k_{r,1} = 6,000 \text{ng} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$</td>
<td>$t_4 = 100 \text{ng/ml}$</td>
<td>$n_4 = 4.5$</td>
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<tr>
<td>SRIF</td>
<td>$k_2 = 2.8/h$</td>
<td>$k_{r,2} = 3,900 \text{pg} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$</td>
<td>$t_2 = 66 \text{pg/ml}$</td>
<td>$n_2 = 5$</td>
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<tr>
<td>GHRH</td>
<td>$k_3 = 2.5/h$</td>
<td>$k_{r,3} = 10,800 \text{pg} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$</td>
<td>$t_5 = 68 \text{pg/ml}$</td>
<td>$n_5 = 40$</td>
</tr>
</tbody>
</table>

Delay constants: $D = 0.75 \text{ h}$; $T = 0 \text{ h}$. Equation for $S_{\text{min}}$: $k_{r,\alpha}S_{\text{min}} = 180 \text{ pg} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$. Michaelis-Menten constant: $t_0 = 1,900 \text{ ng/ml}$. 

Fig. 13. Output of the modified reference model, assuming a direct negative feedback effect of GH on GHRH neurons. The particular parameter set used here is shown in Table 3.
Table 3. Partial sensitivity analysis: individual intervals for some of the parameters in which the model preserves its key features

<table>
<thead>
<tr>
<th>Rate</th>
<th>Constants</th>
<th>Release Constants</th>
<th>Thresholds</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH</td>
<td>$k_1 = 2.7 h$</td>
<td>$k_{r,1} = 5.775 \text{ng ml}^{-1} \text{h}^{-1}$</td>
<td>$t_4 = 47 \text{ng ml}$</td>
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<tr>
<td></td>
<td>($k_2/2; 10k_1$)</td>
<td>($k_{r,1}/6; 2k_{r,1}$)</td>
<td>($t_4/4; 5t_4$)</td>
</tr>
<tr>
<td>SRIF</td>
<td>$k_2 = 5.0 h$</td>
<td>$k_{r,2} = 5.220 \text{pg ml}^{-1} \text{h}^{-1}$</td>
<td>$t_2 = 35 \text{pg ml}$</td>
</tr>
<tr>
<td></td>
<td>($k_2/2; 2k_2$)</td>
<td>($k_{r,2}/6; 2k_{r,2}$)</td>
<td>($t_2/4; 8t_2$)</td>
</tr>
<tr>
<td>GHRH</td>
<td>$k_3 = 8 h$</td>
<td>$k_{r,3} = 76.800 \text{pg ml}^{-1} \text{h}^{-1}$</td>
<td>$t_1 = 504 \text{pg ml}$</td>
</tr>
<tr>
<td></td>
<td>($k_3/1.2; 10k_3$)</td>
<td>($k_{r,3}/12; 12k_{r,3}$)</td>
<td>($t_1/10; 8t_1$)</td>
</tr>
</tbody>
</table>

Michaels-Menten constant: $t_3 = 1.900 \text{ng ml}^{-1}$

(*$*$ Means that increase in this value does not impact significantly the model output. In this particular case, this is due to the fact that increase of $t_2$ releases the suppression of GH by SRIF, but SRIF still depresses the system by decreasing GHRH release, which, in turn, obliterates the GH peak.

interval) is a reflection of the time delay in the effectual onset of GH autofeedback. Conversely, the prolonged interval between successive (multiphase) volleys is a measure of the duration of effectual SRIF actions (i.e., the time required for the escape of GH inhibition of the volley).

Trough elevations of SRIF output are initiated by way of time-delayed GH feed forward on SRIF release. This time delay has been established experimentally by monitoring hypothalamic SRIF secretion and gene expression after either systemic (homologous) or icv (homologous) GH injections (8, 15, 21, 34, 47, 53). The two estimates are comparable (~45 min and 20–80 min, respectively), and each allows in our formulation recurrent 3.3-hour cycles of GH secretion. Elevated SRIF output during the intervolley interval suppresses the release of both GH and GHRH, thus preventing the appearance of GH peaks during a trough. According to this reasoning, the interval between consecutive multiphasic GH volleys is an indirect measure of the duration of GH’s autofeedback on SRIF. In contrast, the intravolley GH interburst interval (time lapse between consecutive GH secretory bursts within a volley) reflects the duration of GH’s autorepression of GHRH secretion. If this perspective is relevant to the human also, then the results of high-intensity blood sampling for GH every 5 min in normal young men that define an intervolley GH interval of ~65–100 min would point to a 65–100 min GH autofeedback time delay in the human (22). This prediction matches the demonstrated effectiveness of GH autofeedback in men of 120 min (41, 42). The corresponding intravolley (interburst) interval was 25–45 min long, suggesting a much shorter duration of GH autofeedback on GHRH release. Indeed, here we successfully model GH autofeedback on GHRH imposing a delay of 7 min on GH’s negative effects. Further studies could explore the range of latencies about 7 min, which could maintain intravolley GH pulses. Reconstruction of unobservable feedback activity in the GH axis would be a valuable application of an objective experimentally based network construct. For example, in relation to the more complex GH dynamics of the female, the presence of high-frequency GH pulses in the absence of recurrent GH multiphase GH volleys would predict limited GH autofeedback on SRIF release, because the latter is here inferred to terminate a volley. This notion has been affirmed empirically by GH infusions in the rat (5, 12, 13, 21, 28). How putative resistance to GH autofeedback on SRIF is mediated more expressively in the female (e.g., via an elevated SRIF threshold to feed-forward drive by GH) is not known. In contrast, the present model allows for preserved GH autoinhibition of GHRH release in the female, which also has been corroborated at the level of GH autorepression in the rodent (18). More particularly, the present model forecasts that GH’s rapid autofeedback on SRIF would sustain the high-frequency GH oscillations in the female if this interpulse interval mirrors the duration of GH’s restraint of GHRH output. Indeed, our present simulations show that SRIF withdrawal can reproduce a high-frequency GH rhythm, apparently due to rapid and reciprocal GH-SRIF interactions during a permissive reduction of SRIF levels (see Figs. 3B and 8). In this regard, when SRIF is putatively reduced during sleep, high-intensity (30 s) overnight sampling of GH in the human reveals very rapid GH oscillations occurring as often as every 12–45 min (23).

Apparent elimination constants for SRIF and GHRH in the present model denote effective in vivo decay of their biological actions. Thus simple disappearance rates from blood of tissue fluids would be more rapid, given the persistence of cellular responses after effector pathway activation. We infer further that the recovery of GHRH secretion after GH-induced suppression is more rapid than the time required for the first GH pulse to elicit SRIF secretion, i.e., intravolley GH oscillations (due to reciprocal GH-GHRH interactions) recur before SRIF stimulation is fully effectual to quench any given volley.

The parameter sensitivity analysis showed that the reference parameter set (Table 1) has some flexibility, consistent with some biological diversity (see Model Reactivity to Specific Parameter Changes: Partial Parameter Sensitivity Analysis). Although it is not computationally feasible to map a full 18-dimensional parameter space, the present parameter choices (see Table 1) are physiologically meaningful. Indeed, a qualitative male pattern emerges, even allowing for a zero lag (direct) negative GH feedback on GHRH (see Fig. 13 and Table 2). Post hoc analysis revealed that the later construct reproduces the key interventional experiments described in Model Reactivity to Defined Interventions and that SRIF is obligatory to sustain GH-GHRH oscillations. Thus the “direct” (0 lag) negative GH feedback on GHRH formulation contains the core autonomous pulse-generating elements.

The present model forecasts significant rebound GH secretion after cessation of short-term SRIF infusion due to hypothalamic GHRH release, as inferred earlier.
in in vivo investigations in the adult male rat (continuous SRIF infusion) (11, 31, 46). The current formulation also predicts high-frequency GH oscillations if endogenous SRIF is withdrawn. In this regard, a recent study in the young rat showed that in vivo administration of a linear hexapeptide antagonist of the SRIF receptor actually augments GH secretion and linear growth (1). This initial paradox might be explained by the present biomathematical network, wherein effectual muting of SRIF actions on GHRH and GH can elicit unabated cyclic GH-GHRH interactions of potentially high amplitude (see Fig. 3B).

Perspectives

The dynamic formulation of the GH axis is presented in terms of two biological oscillator mechanisms, neither of which is autonomous, but rather driven by variably delayed GH-GHRH and GH-SRIF reciprocal interactions. Investigating the specificity of within-axis coupling features may aid in understanding pathophysiology and interspecies differences, assuming that a spectrum of feedback and feed-forward signal strengths and time delays permit stable output. This is not yet established. For example, mechanisms underlying sexually dimorphic and/or age-related contrasts in GH dynamics might be appraised most effectively by complementation of basic laboratory and computer-assisted experiments. Moreover, the present framework may be extended when new physiological data emerge that establish the role(s) of additional GH secretagogues and/or modify existing precepts.

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REFERENCES

28. Maiter DM, Gabriel SM, Koenig JI, Russell WE, and Mathews CK and van Holde KE.
29. Mikawa T, Masui R, and Kuramitsu S.
30. Miki N, Ono M, and Shizume K.