

Unequal autonegative feedback by GH models the sexual dimorphism in GH secretory dynamics

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Farhy, Leon S., Martin Straume, Michael L. Johnson, Boris Kovatchev, and Johannes D. Veldhuis. Unequal autonegative feedback by GH models the sexual dimorphism in GH secretory dynamics. *Am J Physiol Regulatory Integrative Comp Physiol* 282: R753–R764, 2002; 10.1152/ajpregu.00407.2001.—Growth hormone (GH) secretion, controlled principally by a GH-releasing hormone (GHRH) and GH release-inhibiting hormone [somatostatin (SRIF)] displays vivid sexual dimorphism in many species. We hypothesized that relatively small differences within a dynamic core GH network driven by regulatory interactions among GH, GHRH, and SRIF explain the gender contrast. To investigate this notion, we implemented a minimal biomathematical model based on two coupled oscillators: time-delayed reciprocal interactions between GH and GHRH, which endow high-frequency (40–60 min) GH oscillations, and time-lagged bidirectional GH-SRIF interactions, which mediate low-frequency (occurring every 3.3 h) GH volleys. We show that this basic formulation, sufficient to explain GH dynamics in the male rat [Farhy LS, Straume M, Johnson ML, Kovatchev BP, and Veldhuis JD. *Am J Physiol Regulatory Integrative Comp Physiol* 281: R38–R51, 2001], emulates the female pattern of GH release, if autofeedback of GH on SRIF is relaxed. Relief of GH-stimulated SRIF release damps the slower volleylike oscillator, allowing emergence of the underlying high-frequency oscillations that are sustained by the GH-GHRH interactions. Concurrently, increasing variability of basal somatostatin outflow introduces quantifiable, sex-specific disorderliness of the release process typical of female GH dynamics. Accordingly, modulation of GH autofeedback on SRIF within the interactive GH-GHRH-SRIF ensemble and heightened basal SRIF variability are sufficient to transform the well-ordered, 3.3-h-interval, multiphasic, volleylike male GH pattern into a femalelike profile with irregular pulses of higher frequency.

somatostatin; growth hormone-releasing hormone; hypothalamus; mathematical model; male; female; gender; somatotrophic axis

PULSATILE SECRETION OF GROWTH hormone (GH) by the anterior pituitary gland is governed by several core neuromodulators, such as hypothalamic GH-releasing

hormone (GHRH) and the GH release-inhibiting peptide somatostatin (SRIF) (16, 48, 56, 57, 60, 66). Both peptides are carried from hypothalamic neurons via the hypophysial portal circulation to somatotrope cells. GHRH stimulates the synthesis and release of GH, whereas SRIF antagonizes GH secretion. Circulating GH inhibits its own secretion via feed-forward (stimulation) on SRIF and feedback (repression) on GHRH (7, 13, 20, 23, 26, 48, 55, 63). The foregoing basic linkages are sufficient to engender GH pulsatility (16), but it is not known whether such simple connections will reproduce the vividly sexually dimorphic patterns of GH release (20, 22, 23, 47).

The adult male rat maintains 3.3-h-interval, multiphasic GH peaks separated by undetectable GH concentrations (58). The female rat manifests more irregular pulses of higher frequency and lower amplitude superimposed on an elevated baseline. Different patterns of GH output mediate some of the sexual dimorphism in body growth and gene expression in the rodent (23).

Sex steroid depletion and add-back studies in the rat can induce a full spectrum of male- and female-like GH patterns (22, 42, 43). The regulatory basis for such neuroendocrine phenotypes is not known. As one approach to this issue, we have developed a simple networklike model of male GH-GHRH-SRIF interactions (16).

Earlier biomathematical constructs of the GH axis model the typical male pattern (5, 16, 65). For example, Brown et al. (3) explored the kinetics of GHRH actions at the pituitary level. Chen et al. (5) added network features but at the expense of high parameter complexity. Wagner et al. (65) assumed an autonomous GHRH pulse generator to impose repeated pulses within a GH secretory volley. More recently, Farhy et al. (16) used time-delayed feed-forward and feedback connectivity among GH, GHRH, and SRIF to drive volleys of GH

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secretion typical of the adult male rat. However, to our knowledge, none of the foregoing constructs explains the female-specific GH pattern of irregular, low-amplitude and high-frequency GH pulses, high baseline serum GH concentrations, and reduced autonegative feedback by GH (9, 23). The present work addresses the latter mechanistic issue.

METHODS

Primary Sex Differences

There are vivid sex differences in GH secretion in the adult rodent and human (4, 5, 10–12, 22, 23, 25, 26, 36, 38–40, 42, 43, 47, 50, 56). In the rat, the male GH profile consists of 3.3-h-interval multiphasic volleys (58), whereas the female GH pattern has high-frequency (40–60 min) oscillations of irregular amplitude imposed on a readily detectable inter-pulse baseline. Clark et al. (10) further reported occasional intervals of low-amplitude pulses interspersed with rapid high-amplitude peaks in the female. Frequently sampled GH profiles also show multiphasic volleys in the male rodent (4, 10–12, 22, 23, 36, 38–40, 42, 43, 47, 50). In both sexes, more rapid oscillations are variable in amplitude, duration, and frequency (23, 43, 47). Such within-volley variability in the male should be distinguished from the nearly 3.3-h period between-volley intervals. The present model envisions variability due to minimal in vivo fluctuations in feedback and feed-forward within the network.

Core Two-Oscillator GH Network

Our primary GH network is based on five regulatory interactions among GH, GHRH, and SRIF (16): 1) GHRH's drive of pituitary GH release (23, 34, 38); 2) competitive inhibition of GH release by SRIF (9, 34); 3) GH autofeedback by stimulating SRIF with a time delay (6, 44, 51, 69); 4) SRIF's inhibition of GHRH secretion (15, 23, 58); and 5) delayed GH autonegative feedback on GHRH (8, 17, 23,

36–38, 50, 54, 62; Fig. 1). Interactions 3 and 5 give rise to two coupled oscillators (19): 1) the GH-GHRH oscillator and 2) the GH-SRIF oscillator.

We postulate that the GH-GHRH oscillator drives high-frequency (40–60 min) GH pulsatility in both sexes (see *Primary Sex Differences*), whereas the GH-SRIF oscillator (because of the longer delay in feedback) mediates recurrent low-frequency (3.3-h interval) malelike volleys (16). The system mimics observed patterns of GH release in the adult male rat, including GH autonegative feedback and SRIF-induced rebound GH secretion (16).

Connectivity is encapsulated in the following core equations, which describe the rate of change of each hormone with respect to feedback and feed-forward inputs

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

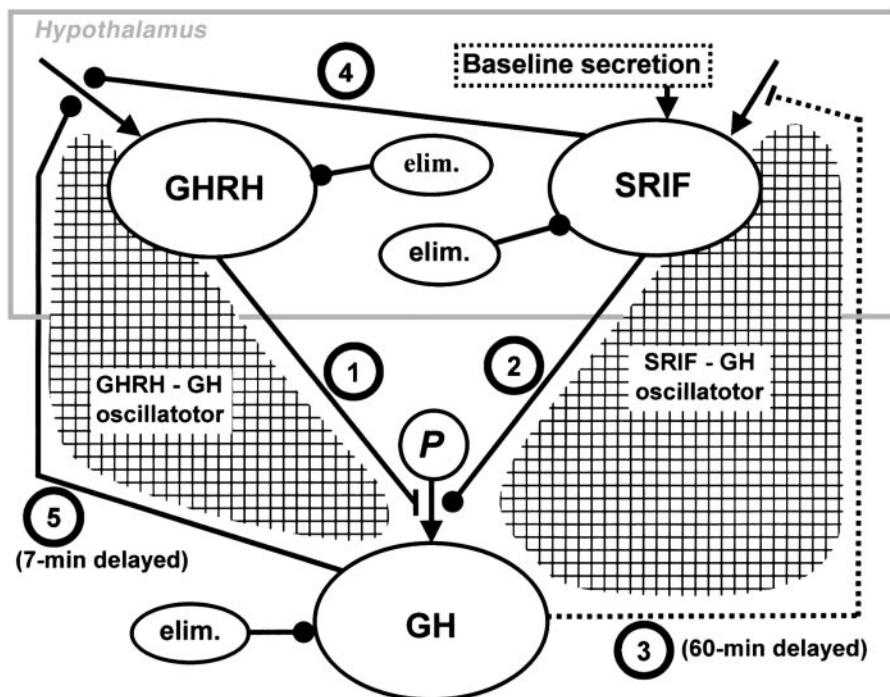
$$SRIF' = -k_2SRIF + k_{r,2} \left[\frac{S_{min} - 1}{1 + GH(t - D)/t_3} + 1 \right] \quad (2)$$

$$GHRH' = -k_3GHRH + k_{r,3} \left[\frac{1}{(SRIF/t_4)^{n_4} + 1} \frac{1}{(GH(t - T)/t_5)^{n_5} + 1} \right] \quad (3)$$

where GH, SRIF, and GHRH denote concentrations of the corresponding peptide; the derivatives (GH', SRIF', and GHRH') are taken with respect to time *t*; *k*₁, *k*₂, and *k*₃ are respective rate constants of elimination; *k*_{r,1}, *k*_{r,2}, and *k*_{r,3} are rate constants of release; *n*₁, *n*₂, *n*₄, *n*₅ and *t*₁, *t*₂, *t*₄, *t*₅ are Hill coefficients and thresholds, respectively, for the corresponding regulatory functions numbered in Fig. 1; *T* and *D* are the time-delay constants for GH's feedback on GHRH and SRIF, respectively; and *t*₃ is the Michaelis-Menten constant defining feedback sensitivity.

If baseline “tonic” SRIF availability to somatotrope cells is viewed as *S*_{min} [replacing the positive constants *k*_{r,2}*S*_{min} and *k*_{r,2}(1 - *S*_{min}) by *S*_{min} and *k*_{r,2}, respectively], then the rate of change of SRIF availability to somatotrope cells (SRIF'), is

Fig. 1. Schema of connections within the growth hormone (GH) feedback network (see Ref. 16 for details). Inhibitory interfaces [including elimination (elim) processes] are denoted by lines ending with solid circles, whereas stimulatory interactions are presented as “T” endings. Interactions are numbered consecutively from 1 to 5, as described in the text (see *Core Two-Oscillator GH Network*). Segments of the network identified by dotted lines were assumed to be sexually dimorphic (see *Sex-Related Specificity of Parameter Set*). Two network-specific oscillators are highlighted. P, pituitary gland; GHRH, GH-releasing hormone; SRIF, somatostatin.



$$\text{SRIF}' = -k_2 \text{SRIF} + S_{\min} + k_{r,2} \left[\frac{\text{GH}(t-D)/t_3}{1 + \text{GH}(t-D)/t_3} \right] \quad (4)$$

where the first term denotes SRIF's decay from the system, S_{\min} corresponds to baseline (GH-independent) SRIF secretion, and the third term defines additional GH feedback-dependent SRIF release. The following minimal system of first-order nonlinear differential equations then reflects the GH network

$$\text{GH}' = -k_1 \text{GH} + k_{r,1} \left[\frac{(\text{GHRH}/t_1)^{n_1}}{(\text{GHRH}/t_1)^{n_1} + 1} \frac{1}{(\text{SRIF}/t_2)^{n_2} + 1} \right] \quad (5)$$

$$\text{SRIF}' = -k_2 \text{SRIF} + S_{\min} + k_{r,2} \left[\frac{\text{GH}(t-D)/t_3}{1 + \text{GH}(t-D)/t_3} \right] \quad (6)$$

$$\begin{aligned} \text{GHRH}' = & -k_3 \text{GHRH} \\ & + k_{r,3} \left[\frac{1}{(\text{SRIF}/t_4)^{n_4} + 1} \frac{1}{(\text{GH}(t-T)/t_5)^{n_5} + 1} \right] \quad (7) \end{aligned}$$

Sex-Related Specificity of Parameter Set

The original parameters in the core model equations emulate the typical adult malelike pattern of GH release (see Ref. 16 for details). Here, we test the hypotheses that femalelike GH patterns reflect blunted SRIF stimulation by GH autofeedback and/or elevated amount and/or variability in SRIF release. Because there is no evidence of sex-related differences in the elimination of SRIF (k_2) or D , these parameters (*Eq. 6*) were not tested. However, selectively elevating S_{\min} (baseline effective SRIF availability) by 2.2-fold attenuated GHRH-induced GH release by 50–65% as inferred earlier experimentally in the female (4) without increasing mean SRIF concentrations (23, 38).

Diminished feedback of GH on SRIF could be modeled by decreasing the rate of SRIF release ($k_{r,2}$) and/or elevating t_3 of feedback sensitivity. No direct experimental data are available to distinguish between these choices in the female [unlike measurements of SRIF responses to a GH bolus in the male rat (6)]. Thus we tested the impact of both a decrease in the constant $k_{r,2}$ (e.g., 15-fold less SRIF release than in the male) and an increase in t_3 (e.g., 5-fold lesser sensitivity). These changes would reduce the efficacy (maximum) and blunt the potency (sensitivity) of GH's upregulation of SRIF release. Smaller changes preserve some delayed-feedback action of GH on SRIF at very high (e.g., pharmacological) GH levels.

The other SRIF-related parameters, viz., the thresholds t_2 and t_4 , govern the onset of SRIF's inhibition of pituitary GH and hypothalamic GHRH secretion, respectively (Fig. 1). A physiological expectation is that GH-induced SRIF release does not block GHRH-driven GH peaks in the female. Altering (increasing) t_2 and t_4 would meet this expectation but would eliminate the known inhibitory effect of SRIF on pituitary GH release in the female (9). Thus

we have not presently explored the effects of altering t_2 or t_4 . Data regarding possible gender differences in t_5 (GH autofeedback on GHRH) are incomplete (8, 37, 52). Accordingly, we have tested the impact of "escape" of GHRH from GH auto repression further (see RESULTS).

In summary, the foregoing reference female model (see Table 1) corresponds to known experimental data in the rat. This construct generates uniform low-amplitude (<70 ng/ml) and high-frequency (~45 min) oscillations on an elevated baseline GH concentration (~30 ng/ml) (see *Basic Female Model Output* in METHODS).

GH Irregularity

Possible explanations for irregular GH release patterns in the female rat (10, 22, 47) include at least the following: 1) variability in the feedback actions of GH on GHRH or SRIF release; 2) variability in T , denoting the time latency of GH's feedback on GHRH, or in D , signifying the time latency for SRIF release induced by GH; and/or 3) variability in the baseline SRIF secretion rate.

Although variability in the system feedback parameters (*modeling options 1 and 2* above) cannot be excluded, preliminary efforts to achieve physiologically realistic GH variability by *modeling options 1 and 2* above were unsuccessful (see RESULTS). Moreover, direct hypophysial portal venous blood-sampling protocols establish considerable variability [~30% coefficient of variation (CV)] in baseline SRIF release over time in individual ovariectomized ewes (J. D. Veldhuis, T. P. Fletcher, K. L. Gatford, A. R. Egan, and I. J. Clarke, unpublished observations). The latter variability is essentially random, based on approximate entropy (ApEn) estimates. Hence, we here allow S_{\min} to vary stochastically with a CV of 30%. For comparison, we explored the impact of the same stochastic input on S_{\min} in the male reference model.

ApEn and Sample Entropy Approach

ApEn (45, 46) and sample entropy (SampEn) (49) are scale- and model-independent statistics to quantify relative orderliness (or process randomness) of time series containing as few as 50–300 samples. For example, ApEn discriminates GH orderliness in the rat in the following rank order (from maximally to minimally irregular): intact female, gonadotrophin-releasing hormone agonist (triptorelin)-treated female, gonadectomized female, gonadectomized male, GHRH agonist-treated male, and intact male (22). Thus we have applied ApEn and SampEn to quantitate relative orderliness of simulated male and female GH patterns. To maintain constant sample series size, we compare equivalent-length male and female GH model-generated profiles; e.g., 240 samples to mimic blood sampling every 5 min for a 20-h period. In the simulations, "observed" profiles generated by the reference male and female models are presented without further perturbation

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

	Rate Constants	Release Constants	Thresholds	Hill Coefficient
GH	$k_1 = 2.7 \text{ h}^{-1}$	$k_{r,1} = 5775 \text{ ng} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$	$t_5 = 47 \text{ ng/ml}$	$n_5 = 3.5$
SRIF	$k_2 = 5 \text{ h}^{-1}$	$k_{r,2} = 350 \text{ pg} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$	$t_2 = 35 \text{ pg/ml}$	$n_2 = 3.5$
			$t_4 = 22 \text{ pg/ml}$	$n_4 = 3.5$
GHRH	$k_3 = 8 \text{ h}^{-1}$	$k_{r,3} = 76,800 \text{ pg} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$	$t_1 = 504 \text{ pg/ml}$	$n_1 = 3.5$

Delay constants: $D = 1 \text{ h}$; $T = 6.912 \text{ min}$. $S_{\min} = 242 \text{ pg} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$. Michaelis-Menten constant: $t_3 = 5,833 \text{ ng/ml}$. GH, growth hormone; SRIF, somatostatin; GHRH, GH-releasing hormone.

or after the addition of 20% Gaussian noise to mimic combined random procedural and assay variability (5, 23, 25).

RESULTS

Basic Female Model Output and GH Irregularities

Simulated GH, SRIF, and GHRH concentration vs. time plots in the reference female rat model (Eqs. 5–7) are presented in Fig. 2A. There are uniform low-amplitude (<70 ng/ml) and high-frequency (~45 min) oscillations of GH superimposed on an elevated baseline (~30 ng/ml).

Figure 2, B and C, illustrates the impact of altered variability in the feedback actions of GH on GHRH and in the delay constant D (see *GH Irregularity* in METHODS). Only a large (7-fold) increase in the GH-on-GHRH action threshold (t_5) elevated GH levels, and blunted GH oscillations (Fig. 2B). Changing the delay constant D also resulted in physiologically irrelevant model output (Fig. 2C): GH peak amplitudes of 200 ng/ml required a fourfold increase in D ($D = 0.5$ h), resulting in lower baseline GH concentrations and prolonged inter-peak intervals (2 h). Therefore, we infer that variability in the feedback actions of GH on GHRH or in the feedback-delay constants (see *modeling options 1 and 2* in *GH Irregularity* in METHODS) does not readily account for the female rat GH pattern (10).

Figure 3 shows the influence of baseline SRIF variability (0.1, 0.3, and 0.5 SD) on GH profiles simulated in the female reference model. No other sources of stochastic variation are included in these plots. Variability in SRIF release about an unchanging mean induced visually evident and stochastically quantifiable irregularity in the female but not the male model

(below). Further analyses used a baseline variability of 0.3 SD to emulate data reported in the ewe (J. D. Veldhuis, T. P. Fletcher, K. L. Gatford, A. R. Egan, and I. J. Clarke, unpublished observations).

In the present model, oscillations in GH and GHRH release in the female evolve as follows. GHRH released into portal blood drives secretion of GH as the GHRH level approaches its action threshold (t_1). The rise in GH concentrations suppresses GHRH release ~7 min (the time delay T) after GH reaches its feedback threshold t_5 . The GHRH concentration then begins to decline because of elimination. Increased GH output continues until GHRH concentrations fall below the stimulatory threshold. Withdrawal of GHRH input allows GH concentrations to decay because of waning secretion and continuing elimination. Diminished GH levels permit a time-delayed resumption of GHRH release. This reciprocal interaction between GHRH and GH produces recurrent peaks in the femalelike secretory profile, as long as excessive somatostatin inhibition is not present (male pattern). Unequal GH pulse amplitudes and interpulse intervals reflect modulation of GHRH action by the variable SRIF baseline (above). Notably, the same perturbation in SRIF baseline applied to the male fails to induce irregularity (Fig. 4).

The latter plots illustrate the further effects of additive Gaussian noise (0.2 SD) on the GH concentrations, whereby we simulate experimental uncertainties.

Model Reactivity to Defined Interventions

Simulated responses to continuous human GH infusions. To mimic the laboratory experiments of Clark et al. (11), we simulated continuous 6-h infusion of human GH in both the male- and femalelike constructs.

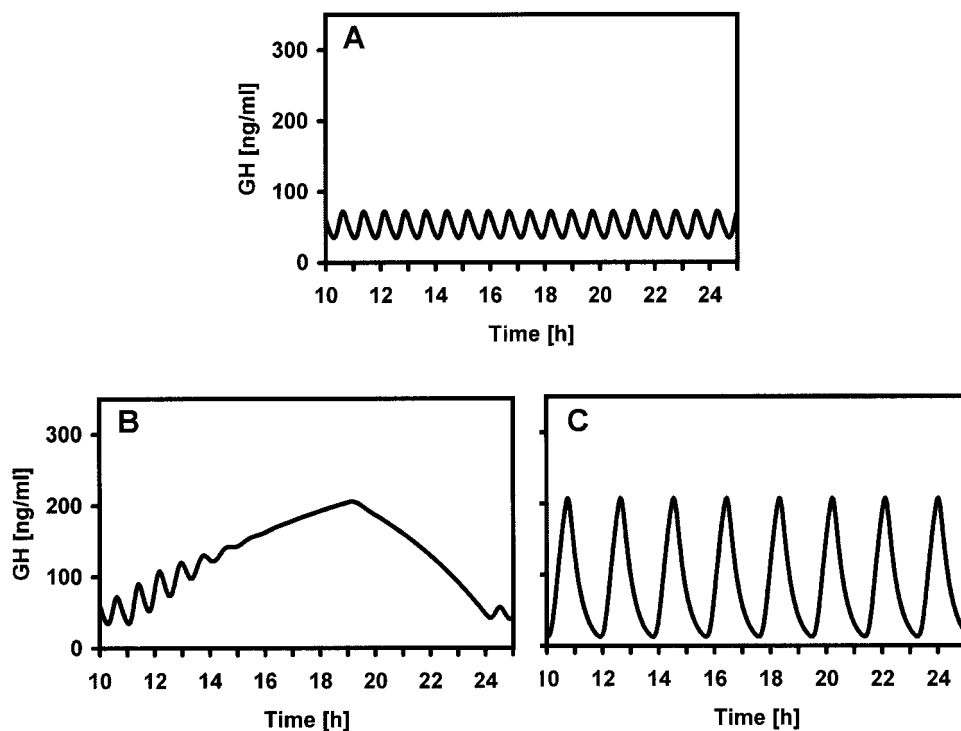


Fig. 2. A: output for the reference female GH model. B: impact of gradually increasing the threshold of GH's inhibition of GHRH. C: effects of prolonging the latency of GH's feedback on GHRH by 4-fold.

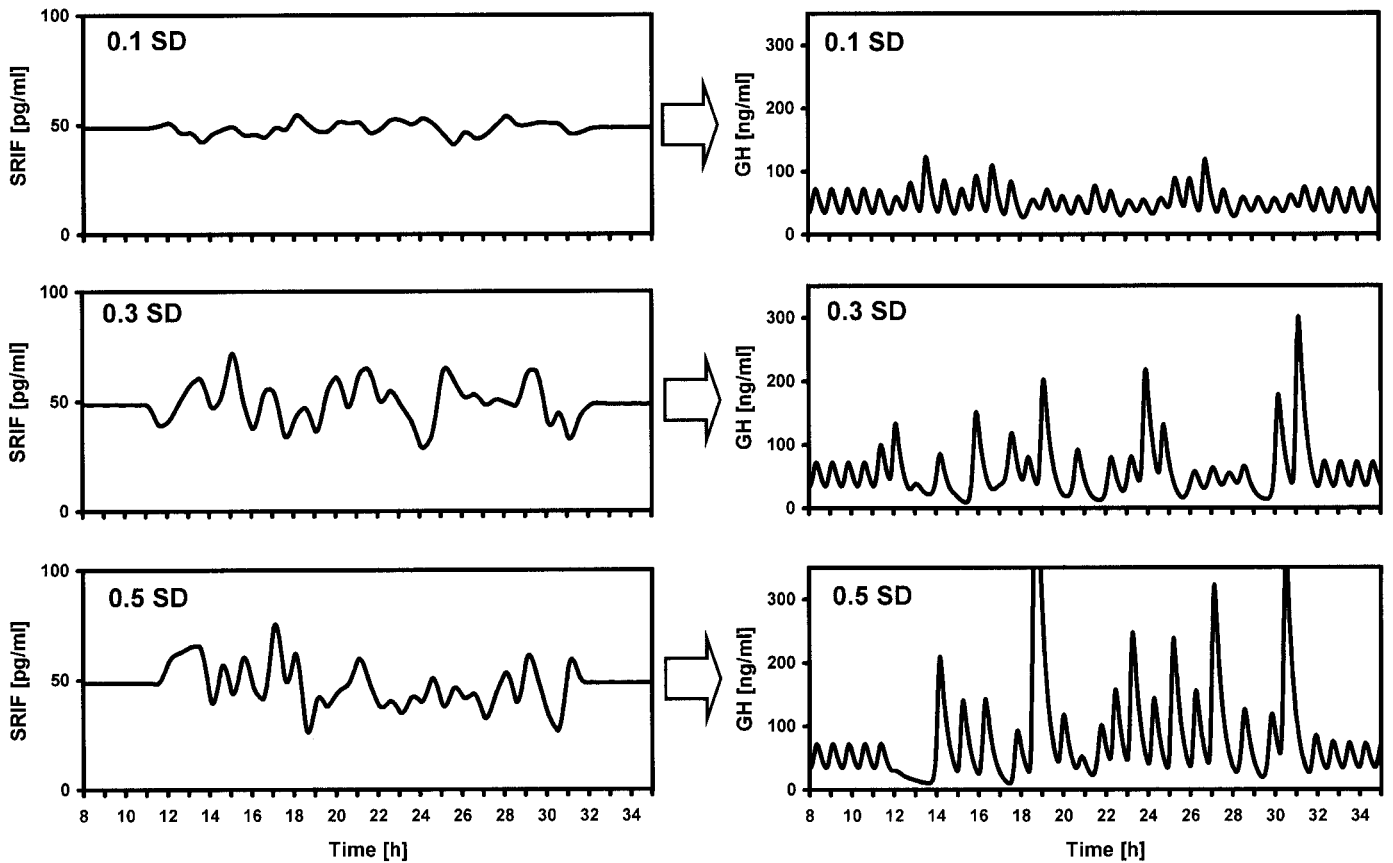


Fig. 3. Reference female GH model responses to 3 levels of stochastic variability of the SRIF baseline (*top*: 0.1 SD; *middle*: 0.3 SD; *bottom*: 0.5 SD) imposed between 1100 and 3200.

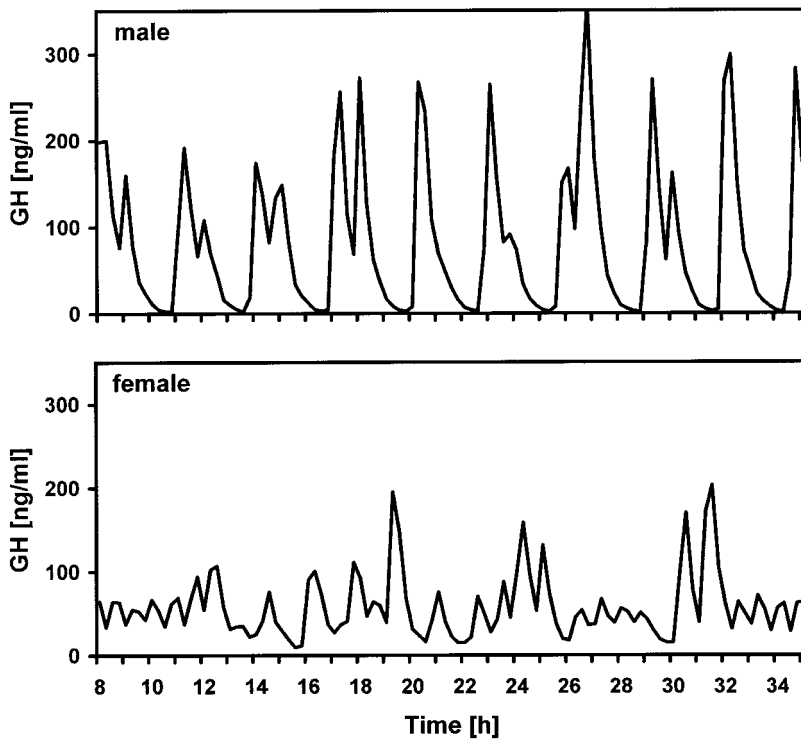


Fig. 4. Contrasting effect of a variable SRIF baseline (time line: 1100–3200; 0.3 SD) on male (*top*) and female (*bottom*) GH model profiles. Procedural uncertainty (20% Gaussian noise) was imposed on the model output.

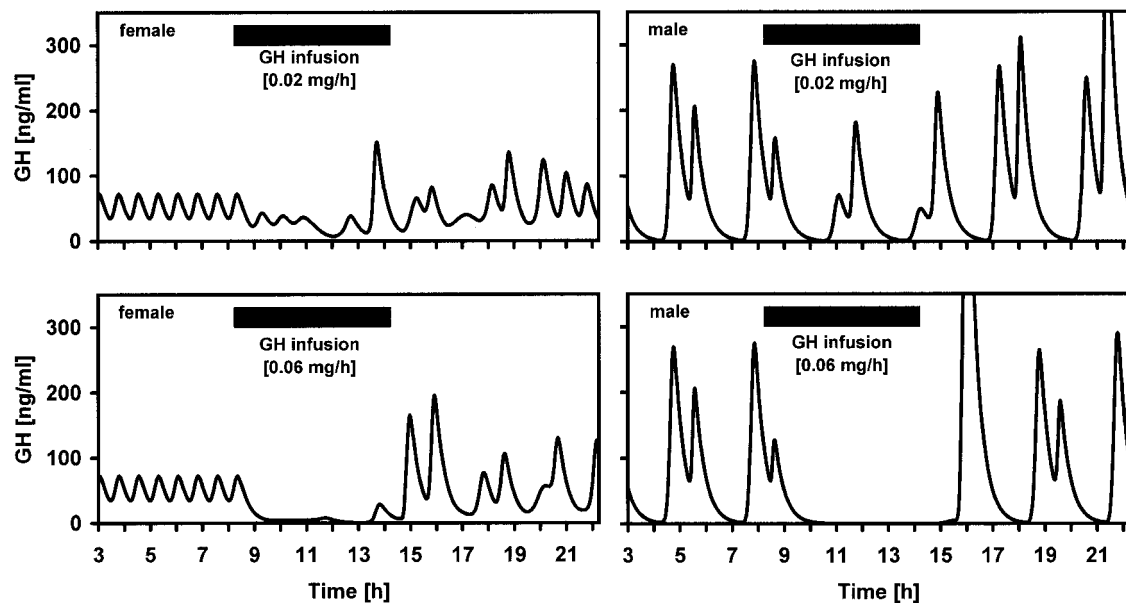


Fig. 5. Comparison between female (*left*) and male (*right*) GH model responses to dose-varying continuous infusions of human GH (time line: 8000–1400). SRIF baseline variability (0.3 SD) was initiated at 1100 (see METHODS).

To this end, we added constant “secretion” of GH in *Eqs. 6 and 7*. The analysis was repeated by imposing a second infusion threefold greater than the first. Variable SRIF baseline release (0.3 SD) was initiated at 1100, 3 h after the simulated GH infusion was begun (8000) (Fig. 5).

Simulated femalelike (Fig. 5, *left*) and malelike (Fig. 5, *right*) GH profiles agree well with published experiments (11), including human GH dose-related inhibition of endogenous GH release. In the female model, suppression of GH release is explained by the negative feedback of GH on GHRH secretion. In the male, GH's

stimulation of SRIF release provides additional restraint of GH output.

Predicted GH secretory-pattern responses to multiple GHRH injections. We tested the response of both the male- and femalelike model to series of 12 simulated injections of GHRH every 45 min to mimic the experimental protocol of Carlsson et al. (4). Simulated infusion of human GH for 6 h was added after the third GHRH pulse (Fig. 6).

The malelike model predicted marked feedback suppression by the GH infusion, as inferred earlier due to GH-induced SRIF outflow during trough periods (see

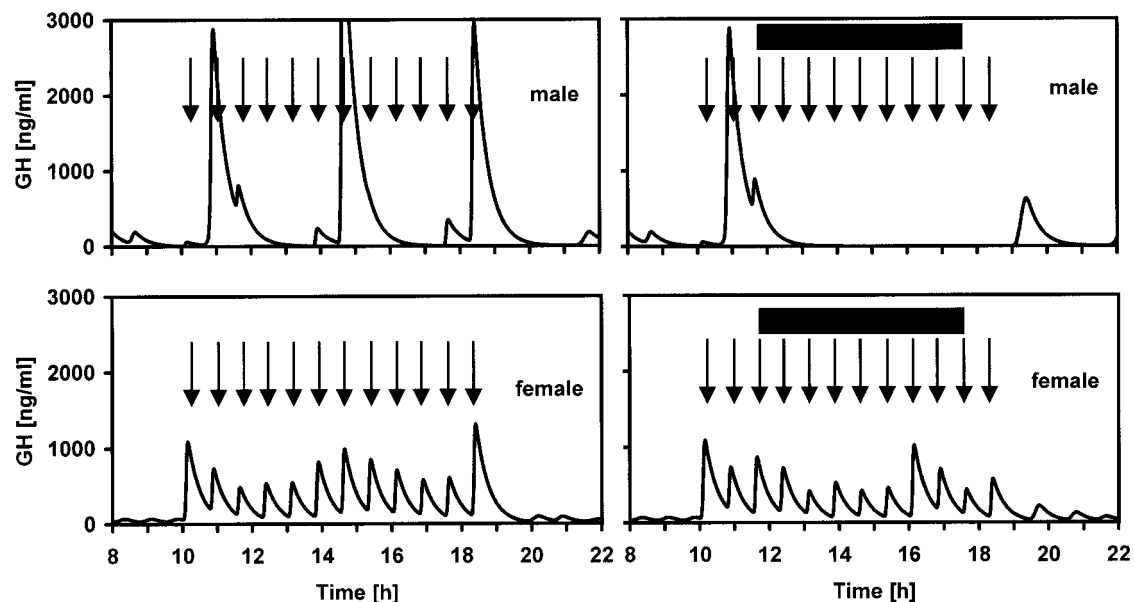


Fig. 6. *Left*: male and female GH model responses to a simulated series of 12 consecutive GHRH injections (arrows) at 45-min intervals starting at 1000 (x-axis). *Right*: added infusion of exogenous GH for 6 h (solid bars) beginning at 1130. Data are presented otherwise as described in the legend of Fig. 5.

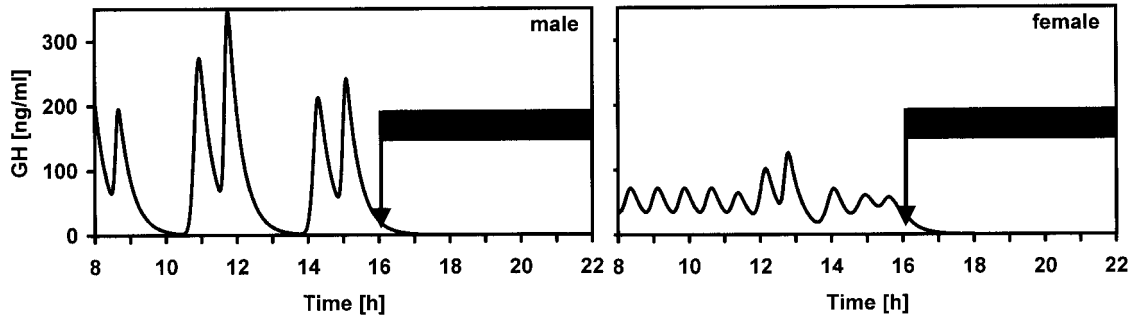


Fig. 7. Model predictions of malelike (*left*) and femalelike (*right*) responses to GHRH antiserum (solid bars) introduced at 1600. Data are presented otherwise as described in the legend of Fig. 5.

Ref. 16 for details) (Fig. 6, *top left*). The extended suppression of GH release during the 6-h exogenous GH infusion in the male (Fig. 6, *top right*) is explained analogously. In contrast, the femalelike model (Fig. 6, *bottom*) predicted continuing GH-secretory responsiveness to repeated injections of GHRH (Fig. 6, *bottom left*). Moreover, exogenous GH infusion did not suppress endogenous GH pulses because of the gender-specific relaxation of GH's feedback stimulation of SRIF release (Fig. 6, *bottom right*). The nonuniform amplitude of GH peaks in femalelike profiles (Fig. 6, *bottom*) mirrors variable baseline SRIF secretion (see METHODS).

Infusion of GHRH antibodies. The model-predicted effect of antiserum to GHRH is illustrated in Fig. 7, wherein we increased the elimination rate of GHRH by 100-fold starting at 1600. Simulated GH profiles agree well with experimental data showing suppression of GH release after the administration of GHRH antibodies in both sexes (39, 66).

Intermittent SRIF infusion. Intermittent SRIF delivery comprised a 9-h simulated SRIF infusion, which

was interrupted for 0.5 h every 3 h (Fig. 8). This analysis assumed that peripheral infusion of SRIF does not directly alter hypothalamic GHRH or SRIF release (16). Analyses were performed without (Fig. 8, *left*) or with (Fig. 8, *right*) simulated continuous infusions of human GH during the third SRIF infusion period (see *Simulated responses to continuous human GH infusions*). Baseline SRIF variability was not imposed here. Corollary experiments (not shown) demonstrated that stochastic variability in S_{\min} did not affect output of the malelike model (because GH-driven SRIF secretion dominates the SRIF baseline variability), whereas the female model displayed large deviations in rebound amplitudes. Simulated GH responses in both sexes agree well with the data reported by Clark et al. (9), wherein concurrent GH infusion abolishes post-SRIF GH rebound in the male but not in the female. The ability of exogenous GH to suppress rebound GH release depends on GH's inhibition of GHRH and stimulation of SRIF release, but the latter action is diminutive in the female (see METHODS).

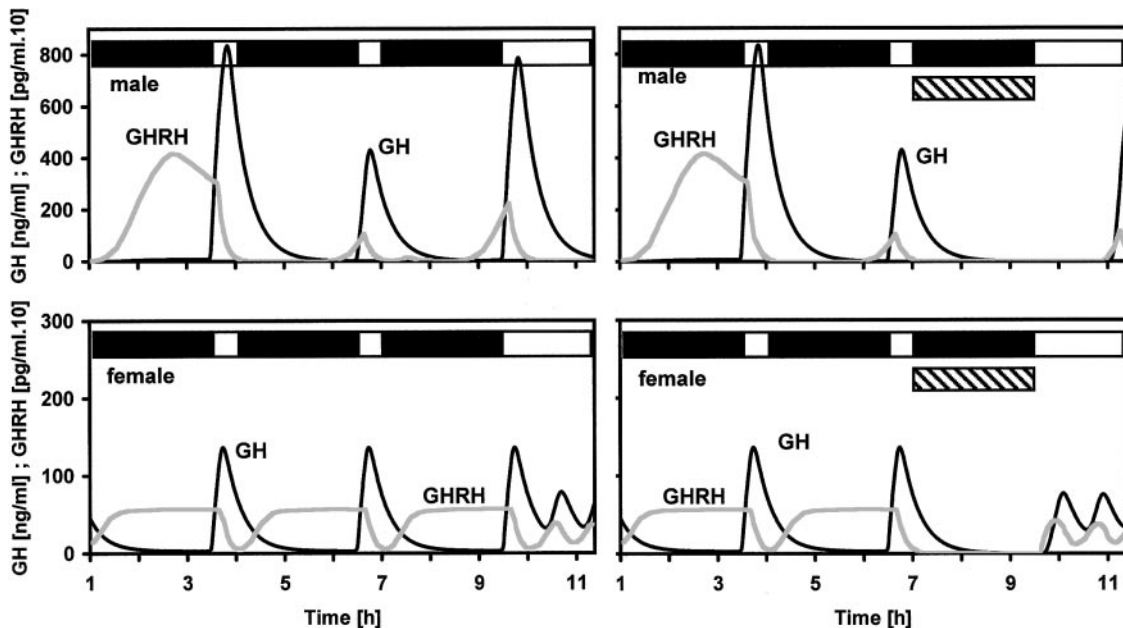


Fig. 8. *Left*: simulation of rebound GH secretion in male (*top*) and female (*bottom*) rats induced by intermittent SRIF infusion (solid bars). *Right*: modeled effect of human GH infusion (hatched bars) on the rebound GH secretion induced by the third SRIF infusion (solid bars) in males (*top right*) and females (*bottom right*) (See METHODS for details).

ApEn and SampEn Analysis

The orderliness of GH time series was quantitated by ApEn and SampEn analyses. Simulated data sets in the male and female comprised 240 data points (with 20% Gaussian noise added), which mimics sampling every 5 min for 20 h. Series were also subjected to first differencing (239 observations) to describe the rate of change of (and detrend) the profiles. Each simulation was repeated 200 times. The resultant mean ApEn and SampEn values are shown in Table 2.

Approximate entropy was quantitated for data pairs (I_1) and triples (I_2) (i.e., parametric choices $m = 1$ and $m = 2$) at a normalized tolerance of $r = 0.2SD$, where SD is the standard deviation of the particular data set (for details see Refs. 45, 46, and 49). Higher ApEn or SampEn denotes greater disorderliness of time series, as evident in the female compared with the male (Table 2). As summarized in the APPENDIX, an analogous procedure was used to calculate the normalized ApEn and SampEn ratios for a transitional sequence of noise-free malelike to femalelike models.

DISCUSSION

The present work illustrates that a simple mechanism of sex-specific regulation of SRIF release could account for female- vs. malelike GH secretory patterns in the adult rat (14, 16, 20, 23, 26, 33, 34, 38, 50, 56–59, 65). This inference arises from a core network of GHRH's feed-forward drive of pituitary GH release, SRIF's competitive inhibition of GH release, GH's stimulation of SRIF after a time delay, SRIF-dependent inhibition of GHRH secretion, and GH's autonegative feedback on GHRH (16). The foregoing minimal connections generate two coupled oscillators, whose output emulates GH patterns in the adult male rat (see the introduction). We extend this formulation first by demonstrating that relaxation of GH autofeedback drive of SRIF outflow induces a femalelike pattern of GH release. The notion of limited GH feedback on SRIF is well documented in this species (11, 12, 23, 26, 36, 38). Reduced autofeedback drive of SRIF outflow in-

duced high-frequency GH oscillations. Mechanistically, recurrent GH pulses are mediated by putative GH-GHRH interactions, whereas loss of 3.3-h-interval volleylike GH release is mediated by the decrease in GH-SRIF connectivity inferred in the female (see METHODS and Fig. 1) (16). Second, we could show that elevating mean baseline SRIF activity in the femalelike model diminishes GH pulse amplitude. Heightened tonic (noncyclic) activity of SRIF is consistent with the results of SRIF-neutralization experiments in the adult female rat and with the ability of estrogen to upregulate pituitary SRIF receptors (31, 64, 68). And, third, we observed that adding variability about mean SRIF release evokes pulse-amplitude irregularity. Variability in central SRIF release is readily apparent in ewes sampled at 5-min intervals for several hours (J. D. Veldhuis, T. P. Fletcher, K. L. Gatford, A. R. Egan, and I. J. Clarke, unpublished observations). Thus all three of reduced GH drive of SRIF outflow, increased SRIF activity and accumulated variability in mean (baseline) SRIF release in the present femalelike model of GH neuroregulation are concordant with physiological data.

Several pivotal experiments have examined mechanistic differences in the neuroregulation of GH patterns in the adult male and female rat in vivo. For example, exogenous GH infusions of sufficiently high dose suppress endogenous GH secretion in both genders (11, 33) but more readily in the adult male than female animal (Fig. 5). In addition, repeated GHRH injections evoke continued GH responses in the female but not in the male rat. Similarly, low-dose infusions of human GH extend GH suppression despite exogenous GHRH stimulation in the male but not the female rat (4). Because GHRH-stimulated GH secretion is abolished in the female by SRIF infusion (4), Clark et al. (11, 12) and Robinson (50) inferred that the female rat is relatively insensitive to GH-induced hypothalamic SRIF release (albeit responsive to SRIF when available) compared with the male. The ability of a high dose of exogenous GH to inhibit endogenous GH output in the female of this species is explained by GH-induced repression of GHRH release (8, 20, 36, 37, 39, 50, 53, 62, 66). The present biomathematical model illustrates the foregoing concept, wherein a reduction of GH's stimulation of SRIF, but not of GH's inhibition of GHRH release, reproduces the femalelike GH secretory pattern and preserves sensitivity to somatostatin.

A higher frequency, and also higher mean amplitude, of GH peaks in the female model was achieved solely by attenuating GH's drive of SRIF release. Elevating average basal SRIF release was required to limit GH pulse height. Effectual SRIF action in the female has been inferred experimentally by SRIF-immunoneutralization experiments (41), the evident suppressibility of GH secretion by exogenous SRIF (4), and estradiol's upregulation of SRIF receptor expression in somatotrope cells and immortalized tumoral cell lines (31, 64, 68). Direct measurements of hypothalamo-pituitary portal venous SRIF release under identical sampling and assay conditions in the female and male

Table 2. Mean ApEn and SampEn values derived from 200 realizations of GH outputs of both the male- and femalelike models

	ApEn		SampEn		Mean SD
	I_1	I_2	I_1	I_2	
	<i>GH concentration</i>				
Male	0.709	0.356	0.294	0.178	109.729
Female	1.019	0.695	0.866	0.723	45.700
	<i>Rate of change of GH concentrations</i>				
Male	1.062	0.515	0.533	0.246	48.840
Female	1.542	0.946	1.448	1.257	22.558

Data correspond to a 20-h period of variable (0.3 SD) baseline SRIF secretion. Procedural uncertainty (20% Gaussian noise) was imposed on the final output of each experiment. Mean values were derived from 200 experiments per model. ApEn, approximate entropy; SampEn, sample entropy; I_1 , data pairs; I_2 , triplets.

will be required to document and distinguish between heightened release and/or action of SRIF in the female rodent.

Exogenous GHRH injection evokes GH release in both sexes (4, 39). In addition, abrupt cessation of SRIF inhibition elicits rebound GH release in both genders (9). The ability of adult female rats to engender occasional high-amplitude GH peaks spontaneously (10) further documents pituitary responsiveness to GHRH. However, other studies allow for reduced GHRH production and/or action in the estrogen-enriched milieu of the rat (20, 23, 26, 37, 38). Although strictly comparable data are not available in humans, recent analyses of the dose-dependent actions of randomly ordered single injections of recombinant human GHRH-(1–44)-amide in postmenopausal women show enhanced GHRH potency (but not efficacy) in response to short-term estrogen repletion (2). In addition, altered actions of GHRH in the female could be mediated by reciprocal changes in basal SRIF outflow. The latter mechanism might also contribute to species differences. For example, where GH and GHRH peak amplitudes are smaller in the female than male rat (Fig. 9, *panels 1 and 6*), GH pulse heights are larger in premenopausal women than comparably aged men (62). Further studies will be

required to assess whether reduced GHRH drive or heightened basal SRIF release maintains low-amplitude GH pulsatility in the female.

Random variability in (constant mean) baseline SRIF release triggered marked pulse-to-pulse amplitude variability in the femalelike (but not malelike) feedback model (Fig. 4). Irregular SRIF signaling could reflect nonuniform secretion and/or delivery of SRIF to somatotropes as well as inconsistent pituitary responsiveness (28, 29). The degree of stochastic variation in basal SRIF outflow postulated here (i.e., a 30% CV) is observed in hypophysial portal blood in the ewe (Ref. 21; J. D. Veldhuis, T. P. Fletcher, K. L. Gatford, A. R. Egan, and I. J. Clarke, unpublished observations). However, further studies will be required to monitor SRIF oscillations in the female of various species and in potential pathophysiology (23).

Extensive experimental data (23) document that the GH-GHRH-SRIF network is subject to multiple internal and external influences [e.g., insulin-like growth factor (IGF)-I, IGF-II, metabolic factors (e.g., glucose, free fatty acids, acidosis), glucocorticoids (e.g., gonadal sex hormones, thyroid hormones, catecholamines, and gastrointestinal and neuropeptides)]. We did not include these factors in the present minimal model, because

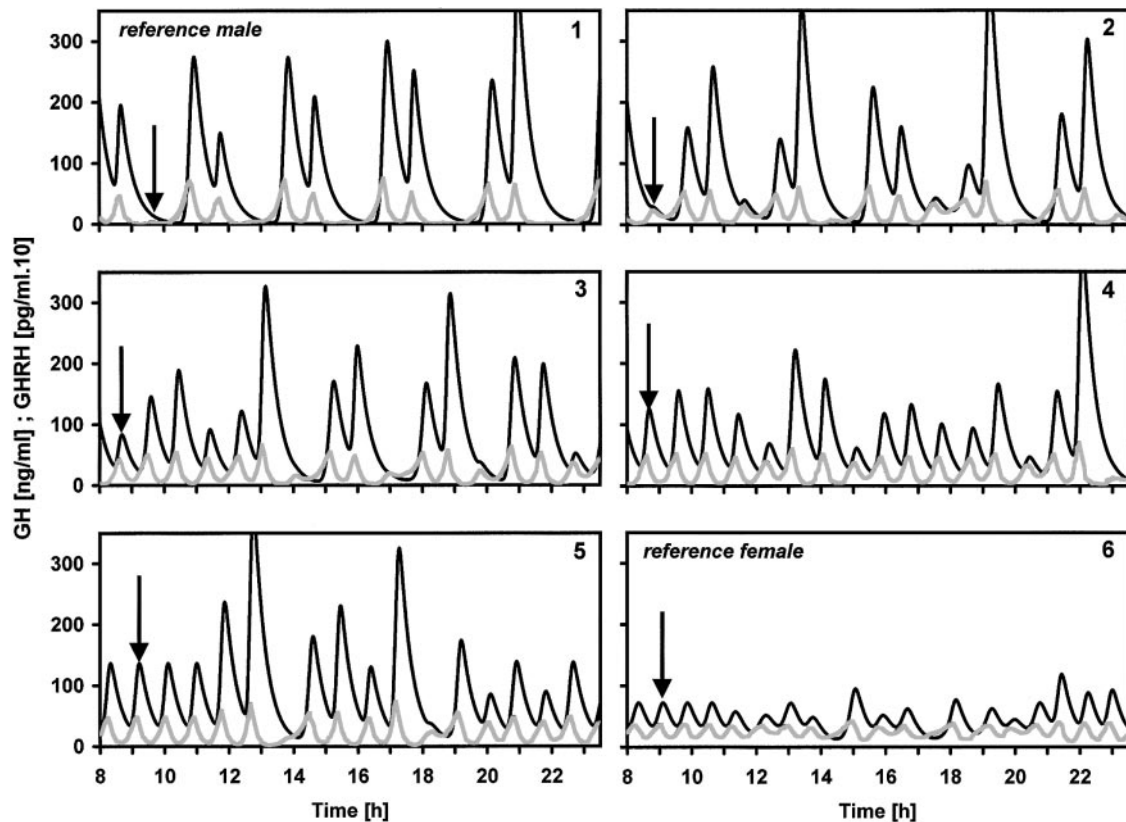


Fig. 9. Sensitivity analysis illustrated for 1 of the 200 realizations each of malelike (*panel 1, top left*) to femalelike (*panel 6, bottom right*) parameter transitions (see Table 3). Parameter evolution consisted of gradually relaxing the feedback of GH on SRIF and elevating baseline SRIF release with constant variability. The arrows (before 1100) track the emergence of a single GHRH (light line)-GH (dark line) spike within the initially malelike interval with femalelike parameter evolution. Data are presented otherwise as described in the legend of Fig. 5. Objective quantitation of regularity changes was performed via approximate entropy and sample entropy analyses on each of 200 simulations carried out for each parameter set shown (see Table 4).

Table 3. Nominal value of parameters used in the transitional models depicted in Fig. 9

	Experiment No.					
	1 (Reference male)	2	3	4	5	6 (Reference female)
t_3	1,166	2,566	2,846	3,033	3,966	5,833
$k_{r,2}$	5,250	3,780	3,486	3,290	2,310	350
S_{\min}	110	149	157	162	189	242

none (with the exception of ghrelin, IGF-I, and IGF-II) has been proven to participate in the GH network in a reciprocal feedback fashion. Further model extension may allow more complex input by such extrinsic regulators. For example, it will be important to obtain detailed data on feedback time dependencies of the actions of IGF-I and IGF-II (23). One could speculate that a prolonged delay in IGF-I feedback on GH release might contribute to the daily rhythmicity of GH release in the female rat, as observed in some studies (10). Thus additional experimental interventions will be important to distinguish both rapid and delayed regulation by other negative and positive inputs to the core GH-GHRH-SRIF system.

Perspectives

Dynamic output of the GHRH-SRIF-GH network presumptively reflects unique nonlinear dose-responsive relationships that link the primary system components. More formal feedback and feed-forward models corroborate this intuition (28–30, 47). According to the present male-female comparisons coupled oscillators within a complex network endow greater orderliness, e.g., for male GH profiles. Thus the malelike GH axis is dominated by delayed feedback of GH on SRIF (which oscillator entrains regular 3.3-h-interval volleys of GH release) and concomitant feedback of GH on GHRH (which inferred oscillator drives rapid GH pulses within volleys). Superimposing variability on an unchanging mean SRIF baseline in this stable feedback construct fails to disrupt the primary rhythmicity conferred by reciprocal GH-SRIF interactions. However, blunting of GH autofeedback on SRIF in the femalelike model unleashes the more rapid GH oscillator system mediated by recurrent GH-GHRH interactions. In this context (but not in the male model), adding minimal stochastic variability to basal SRIF release created marked irregularity of GH release. Stochastic properties in basal GH secretion could emerge from the topographic and functional dispersion of SRIF-secreting neurons in hypothalamic periventricular nuclei. In addition, nonuniform access of SRIF to and/or variable responsiveness among somatotrope cells could mimic irregular SRIF release per se. Whether sex steroids affect one or more of the foregoing processes is not known. However, this consideration is plausible based on analyses of other neuronal systems, such as oxytocin and gonadotrophin-releasing hormone neurons (30, 32, 35). Accordingly, the present network-based formalism points to selected new experiments, which may

aid in clarifying the mechanistic basis of the sex difference in GH neuroregulation.

APPENDIX

Parameter Sensitivity in the Male- and Femalelike Models

The male and female models differ by way of three coefficients: S_{\min} , t_3 , and $k_{r,2}$. Accordingly, we have examined the spectrum of simulated GH patterns across the foregoing three-dimensional parameter space (Fig. 9).

The particular parameter grid for each of the six experiments is shown in Table 3.

Of interest is the gradual transformation of the male-like 3.3-h-interval volley pattern into a femalelike low-amplitude output dominated by high-frequency GH-GHRH oscillations. Irregularity of amplitude modulation is induced by imposing variability on the SRIF baseline (after 1100). In the male-predominant model (Fig. 9, panels 1 and 2), intervalley GHRH release and diminutive GHRH-driven GH pulses are suppressed by strong GH autofeedback-dependent time-delayed SRIF release. The femalelike pattern unfolds progressively in response to selective muting of GH feedback on SRIF. The arrows in Fig. 9 mark emergence in the femalelike parameter space of a single increasingly prominent GHRH pulse and corresponding GHRH-stimulated GH pulse that is undetectable in the male-predominant parameter space (albeit inferable from an initially diminutive rise in GHRH

Table 4. Mean ApEn and SampEn ratios derived from 200 realizations of 6 transitional models (see Table 3 for the particular parameter choice) ranging from malelike (experiment 1) to femalelike (experiment 6)

Experiment No.	ApEn Ratio		SampEn Ratio		Mean SD
	I_1	I_2	I_1	I_2	
<i>GH concentration</i>					
1 (Male)	0.343	0.233	0.160	0.099	92.640
2	0.416	0.342	0.293	0.178	70.195
3	0.434	0.359	0.321	0.194	66.247
4	0.449	0.366	0.339	0.202	63.241
5	0.504	0.393	0.406	0.253	52.828
6 (Female)	0.496	0.432	0.414	0.289	38.357
<i>Rate of change of GH concentration</i>					
1 (Male)	0.390	0.243	0.169	0.095	38.957
2	0.506	0.347	0.283	0.180	31.753
3	0.525	0.366	0.301	0.197	30.952
4	0.538	0.372	0.309	0.204	30.359
5	0.606	0.384	0.395	0.251	26.101
6 (Female)	0.618	0.401	0.474	0.274	17.344

Normalized values were calculated by using 300-fold random reshuffling of the original data set.

concentrations). To quantitate changing GH time-series regularity, we calculated normalized ApEn and SampEn ratios for 200 realizations of the 20-h period after 1100 (see *ApEn and Sample Entropy Approach*) in each of the six transitional models. Mean values are given in Table 4.

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