Pulsatile growth hormone secretion persists in genetic growth hormone-releasing hormone resistance

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Growth hormone secretion is regulated by GHRH-releasing hormone (GHRH), somatostatin, and possibly ghrelin; it may also reflect intrinsic oscillations in somatotropes. The pulsatile pattern of GH secretion and its relation to GHRH and somatostatin rhythms has been evaluated extensively in a number of species (23), and a concept of GHRH-induced GH pulses, modulated by prevailing somatostatin tone and rapid somatostatin oscillations, has been formulated (65). A role of ghrelin in this interplay can be postulated but remains unproved. This model is supported by studies using immunoneutralization of GHRH and somatostatin (65, 80), GHRH, or somatostatin antagonists (5, 33, 34, 73), as well as direct pituitary portal sampling in experimental animals (18, 20, 55, 66). A reciprocal relationship between GHRH and somatostatin, where GHRH pulses coincide with somatostatin troughs, has been reported in rats (37, 55, 65), but this pattern is less clear in sheep or pigs, where more complex relationships between oscillations in GHRH, somatostatin, and GH prevail (8, 15, 20, 66). Thus important species as well as sex differences exist in the regulation of GH secretion. Additional complexities are imposed by nutritional status, sex steroid milieu, and feedback by insulin-like growth factor (IGF) I and GH. In humans, where hypophysial-portal sampling is not feasible, the roles of GHRH and somatostatin have been inferred from studies with continuous, presumably saturating infusions of GHRH, GHS, and somatostatin or its analogs (7, 13, 32, 35, 61, 72, 79) as well as by the use of a GHRH antagonist (33, 34). These studies have provided evidence for a predominant role of GHRH and an additive role of somatostatin in GH pattern regulation in humans. However, despite many years of ingenious investigations, the precise relative contributions of the hypophysiotropic factors to minute-to-minute GH secretion remain difficult to dissect in the intact organism.

One strategy used to gain a better understanding is to selectively remove one factor or its input from the

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system. This has been partially achieved by immunoneutralization studies (65, 80) or treatment with antagonists against GHRH (33, 34, 73) or somatostatin (5). However, pharmacological approaches often have limitations because of incomplete efficacy or lack of complete specificity. Genetic approaches frequently yield more definitive results because of their greater specificity. The recent identification of genetic GHRH receptor (GHRH-R) deficiency in humans presents an opportunity to examine GH secretion in the selective and complete absence of GHRH input.

We (4, 46) and others (50, 78) recently reported a syndrome of GH-deficient dwarfism resulting from a nonsense mutation in the GHRH-R gene (dwarfism of Sindhi). This null mutation is predicted to truncate the GHRH-R near its amino terminus. In its homozygous state, the mutation causes severe, isolated GH deficiency with inability to respond to GHRH and several other GH provocative stimuli, including hexarelin (4, 45, 46, 50, 78). Affected patients also have pituitary hypoplasia, presumably due to lack of normal somatotrope development (49, 50). Other inactivating mutations in the GHRH-R gene exhibit a very similar phenotype (29, 31, 59, 60, 64). The murine homolog is the little (lit/lit) mouse, which harbors an inactivating missense mutation resulting in GH deficiency (14, 16, 24, 41). In all of these cases, GH production is inadequate to sustain normal somatic growth, thereby proving the crucial nature of GHRH for a functioning GH-IGF axis. However, little is known about the degree of residual GH secretion and its temporal pattern. We have employed this unique model of complete GHRH resistance at the GHRH-R to examine residual GH secretion in the absence of GHRH-R input, thereby gaining new insight into the roles of somatostatin and perhaps ghrelin (or its analogs) in generating pulsatile GH secretion.

SUBJECTS AND METHODS

Four adult males (patients 4, 5, 23, and 35 in Ref. 46) with a homozygous GHRH-R defect, aged 23, 28, 28, and 30 yr, respectively, participated in the study. Their genetic, physical, and endocrine characteristics have been described in detail previously (4, 45, 46, 49). None had ever been treated with GH or any other form of endocrine therapy. They traveled from Pakistan to Chicago and were admitted to the Northwestern University General Clinical Research Center (GCRC). After acclimatization for 3 days, the studies were initiated. The study protocol was approved by the Northwestern University Institutional Review Board, and the patients gave informed consent. An intravenous cannula was placed in a forearm vein at 7 AM, and starting at 8 AM, blood (1 ml) was drawn every 10 min over a 24-h period. Blood samples were immediately added to EDTA-containing 2-ml Vacutainer tubes, cooled on ice, and centrifuged within 30 min. The volume of the EDTA solution in the Vacutainers was measured as 36.2 ± 0.28 μl (mean ± SE; n = 9). Plasma was kept frozen until assayed. The patients remained ambulatory at the GCRC, ate their meals (Pakistani food) at regular times, and were allowed to sleep without disturbance during the night. All patient activities were recorded by the nurses on a continuing basis. Blood sampling during sleep was performed from outside the room via long tubing. Blood contained in the dead space of the tubing was reinjected. All patients tolerated the procedure well, and no adverse effects were observed.

GH was measured at the University of Virginia by a sensitive chemiluminescence assay (9) with a limit of detection of 0.01 μg/l and a coefficient of variation ranging from 3.9 to 8.8% in the measurement range relevant for this study. All samples derived from the four 24-h profiles were measured in a single assay to minimize variability due to technical reasons. No correction was made for the plasma dilution (−3%) by the EDTA solution derived from the Vacutainers.

To assess a potential contribution of ghrelin (or a similar endogenous GHS receptor ligand) to fluctuations in plasma GH levels, we also reassayed GH levels by chemiluminescence assay in plasma specimens obtained during a previous study (46) that had examined the effect of hexarelin in the same four patients. Those samples had been stored at −20°C since the original assay.

To determine the degree to which intrinsic assay variability (technical variation) contributes to apparent pulsatility, we assayed a plasma pool (GH level 0.038 μg/l) 136 times in duplicate, arranged the results into a pseudo-time series akin to a circadian profile, and subjected the pseudo-series to the statistical tests used for the circadian profiles. Technical oscillations in this pseudo-series were found to be random and were limited to ≤0.01 μg/l. The pseudo-series also showed an absence of trends, such as assay drift, and absence of features characteristic of hormone secretion such as progressive increases/decreases in sequential samples.

Statistical analysis of the 24-h profiles was performed by cluster analysis, an objective pulse detection program (74), deconvolution analysis, a modeling program designed to dissect various aspects of hormonal time series such as secretion rate and clearance (75, 76), and the approximate entropy (ApEn) statistic, an estimate of underlying process irregularity (52, 54) [deconvolution and ApEn (1, 20% SD) analyses were kindly performed by J. Y. Weltman and Dr. S. M. Pincus, respectively]. The deconvolution method was used to derive pituitary secretion parameters and GH half-life. Pulses with amplitudes of ≥0.01 μg/l (and derived parameters) were ignored, because they have been shown to represent primarily technical variation (12) (for exceptions, see legend to Fig. 1). Minor pulsations within a larger pulse were considered to be part of that composite pulse (28). Differences between daytime and nighttime GH secretion were assessed by comparing 1) mean overall GH levels and 2) mean nadir GH levels between the first and last 12 h of the sampling period (8 AM–8 PM vs. 8 PM–8 AM), or between 8 AM and sleep onset and sleep onset and 8 AM. Areas under the curve in GH stimulation tests were determined by the trapezoidal rule. Summary data are expressed as means ± SD. Comparisons between patient and normative data were made by t-test or by the Mann-Whitney ranked sum test where data were not normally distributed or variances were unequal.

RESULTS

The four circadian plasma GH profiles are shown in Fig. 1A. All four patients had extremely low overall GH levels compared with normal subjects. Their 24-h mean GH levels were 0.052, 0.020, 0.021, and 0.046 μg/l, respectively (Table 1). This was consistent with their phenotype of isolated GH deficiency and their lack of response to several provocative stimuli for GH. However, it is also apparent that plasma GH levels fluctuated in a manner qualitatively similar to that in nor-
Fig. 1. Diurnal growth hormone (GH) secretion in patients with GH-releasing hormone receptor (GHRH-R) deficiency. A: plasma GH concentration profiles (10-min sampling intervals). Note the low numbers on the ordinates and their different scales. B: GH secretion profiles, derived from plasma GH concentration profiles by deconvolution. Horizontal bars denote observed sleep periods. The GH secretion profile for patient 2 is drawn as a dashed line because of the marginal reliability of deconvolution at these low secretion levels (see text). We did not derive numerical deconvolution parameters in Table 1 for that reason. Nevertheless, the GH concentration profile of patient 2 is visually suggestive of secretory episodes and enhanced GH secretion at night, and both the approximate entropy (ApEn) values and night-day comparison of overall and nadir GH levels show highly significant differences from a random oscillatory profile (see text and Table 1). It is for this reason that we show the secretion profile as a tentative graph for the reader’s consideration. In this case, we waived the \( \leq 0.01 \mu g/L \) increment/decrement criterion for designating a peak and let the deconvolution program decide what constitutes a secretory episode. It should be noted that the deconvolution program did not identify any pulses in the pseudo-time series derived from the replicate assay of a single plasma sample, even when increment/decrement criteria were set to zero.
GH secretion in GHRH receptor deficiency

Table 1. GH secretion characteristics, as derived from deconvolution and ApEn analyses

<table>
<thead>
<tr>
<th>Patient</th>
<th>24-h Mean GH (μg/l)</th>
<th>Peak Frequency, no./24 h</th>
<th>Mass Secreted/ Burst, μg/l</th>
<th>Peak Half-Duration, min</th>
<th>t1/2, min</th>
<th>GH Production Rate µg/l</th>
<th>Basal GH Secretion Rate 1.24 h⁻¹</th>
<th>ApEn</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.052</td>
<td>18</td>
<td>0.081</td>
<td>27.1</td>
<td>14.6</td>
<td>3.41</td>
<td>1.95</td>
<td>1.006</td>
</tr>
<tr>
<td>2</td>
<td>0.020</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1.708</td>
</tr>
<tr>
<td>3</td>
<td>0.021</td>
<td>16</td>
<td>0.016</td>
<td>26.1</td>
<td>16.1</td>
<td>1.42</td>
<td>1.17</td>
<td>1.133</td>
</tr>
<tr>
<td>4</td>
<td>0.046</td>
<td>12</td>
<td>0.042</td>
<td>30.3</td>
<td>15.7</td>
<td>2.95</td>
<td>2.44</td>
<td>1.287</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.035 ± 0.02</td>
<td>15.3 ± 3.1</td>
<td>0.046 ± 0.033</td>
<td>27.8 ± 2.2</td>
<td>15.5 ± 0.8</td>
<td>2.59 ± 1.04</td>
<td>1.85 ± 0.64</td>
<td>1.284 ± 0.305</td>
</tr>
</tbody>
</table>

Normal young men (mean ± SD)

Thorner et al. 1.39 ± 0.77 19.0 ± 4.7 3.82 ± 1.99 26.8 ± 6.7 16.5 ± 4.2 73.3 ± 36.1 3.34 ± 2.81 0.240 ± 0.115

Ref. 28 1.78 ± 0.79 12.0 ± 1.2 7.13 ± 2.31 24.8 ± 8.3 17.3 ± 5.9 80.2 ± 37.9 ND ND

P values patients vs. Thorner et al. (Ref. 53 for ApEn)

P = 0.005 0.231 0.014 0.797 1.00 0.007 0.275 1.6 × 10⁻⁵

GH, growth hormone; ApEn, approximate entropy; ND, not determined. Deconvolution data on patient 2 are not shown because his GH secretion rate was too low for reliable determination (see text). GH production rate includes both basal and pulsatile components. Thorner unpublished data in 11 normal lean, male subjects, ages 20–28 yr, body mass index (BMI) 21.1–26.4 kg/m², with the identical GH assay and 10-min sampling intervals represents the best normative match to the present study in terms of subject characteristics and methodology. Hartman et al. (28) reported data in 12 lean males, age 22–28 yr, BMI 21–29 kg/m², with a less sensitive immunoradiometric assay and 10-min sampling intervals. Pulse frequency is in part dependent on assay sensitivity, because pulses below the detection limit of the assay go undetected. For this reason, frequencies tend to be greater with more sensitive assays. For example, van den Berg et al. (71) reported 19.9 ± 4.5 peaks/24 h in middle-aged men by use of an immunofluorometric assay with a sensitivity of 0.0115 μg/l.

This included peaks occurring at 1- to 2-h intervals, higher overall levels at night, a major nocturnal secretory pulse, and the composite nature of secretory episodes consisting of sequential “volleys.” Pulses were detected by both the cluster and the deconvolution programs with a high degree (89.5%) of concordance. There was no apparent relation of GH pulses to eating or any other recorded activity (ambulation, talking to staff, watching television, etc.). In all four patients, nocturnal overall plasma GH levels were significantly higher (P < 0.001) than daytime GH levels regardless of whether nocturnal was defined as commencing at 8 PM or at sleep onset. Similarly, in all patients, the nocturnal nadir GH levels were significantly higher (P < 0.001) than those during the day, with the use of either of the two definitions for nocturnal. In contrast to the patients’ circadian profiles, neither cluster nor deconvolution analysis detected any pulses in the pseudo-time series derived from the replicative assay of a single plasma sample.

Figure 1B depicts the deconvoluted GH secretion profiles. The higher secretory activity during the night is apparent. Two patients showed no detectable GH secretion during the daytime, and in one (patient 2), even the nocturnal secretion was so low that reliable quantification was questionable. We omitted those data/time periods from the overall analysis. Because of this limitation, pulse frequency data in three of the four patients are based on partial circadian (primarily nocturnal) profiles.

Table 1 summarizes the numerical data descriptive of these circadian profiles and the deconvolution and ApEn results. In one patient (patient 2), plasma GH values were near the detection limit throughout the day, a fact that rendered deconvolution difficult. In another (patient 3), levels were similarly low during the daytime, imposing the same limitation for the first half of the diurnal profile. Nevertheless, the remaining information showed that there are peaks occurring on average every 94 min, similar (P = 0.231) to that in normal young (20–28 yr old) male subjects (every 76 min) when examined by the same ultrasensitive assay (M. O. Thorner, unpublished data) or in middle-aged (47 ± 3.5 yr old) males (every 72 min) when an equally sensitive immunofluorometric assay (detection limit 0.0115 μg/l) was used (71). GH plasma half-life (15.5 ± 0.8 min) was also similar to that in normal subjects (17, 28). The GH production per pulse was 45- to 450-fold lower than normal, and the daily GH production rates were 1.8–6.2% of the average GH production rate for normal males in the same age group (28). Basal (i.e., apulsatile) secretion accounted for a high proportion (95%) of the total GH secretion, in contrast to normal men, where ~95% of daily GH production is derived from secretory pulses (71). It should be recognized that the numerical values listed here and in Table 1 are based on only three deconvolution profiles and are partly derived from GH measurements near the assay detection limit. They should, therefore, be considered approximations.

ApEn values were intermediate between zero and maximal (~1.844 for this data series length), implying GH dynamics that were neither flat nor random (patient 2 approached maximal ApEn values, implying a high degree of randomness). Furthermore, ApEn values were higher in all four subjects than in normal males, indicating a greater degree of disorderliness, or lack of quiescence between peaks.

The observed pulsatility in the absence of a GHRH signal raises the question of what drives this pulsatility. To gain information about the potential involvement of ghrelin or a related factor, we reexamined by ultrasensitive chemiluminescence assay the response of these same patients to hexarelin, previously determined only by conventional GH assay (45). All four patients showed some, albeit very minor, GH responses to hexarelin (Fig. 2). This finding is consistent with the responses to GH-releasing peptide-2 reported for pa-
GH secretion in GHRH receptor deficiency

The present study was designed to evaluate GH secretion patterns in vivo in the selective absence of GHRH input to the somatotrope, secondary to genetic GHRH-R deficiency. The study demonstrates that, under these circumstances, some pulsatile GH secretion persists, albeit at greatly decreased amplitudes. Also preserved is another feature of GH secretion, namely enhanced GH secretion during the night, with a dominant sleep-related GH pulse. Rhythmicity of GH secretion dynamics is not only visually apparent but was also detected by three objective, independent methods: cluster, deconvolution, and ApEn. Without GHRH input, the amplitude of GH secretory spikes is two to three orders of magnitude lower than normal, resulting in severe clinical and biochemical GH deficiency (1, 4, 29, 46, 50, 78). The sensitive GH assay employed permitted robust GH measurements in two patients throughout the day and in an additional patient at night (100% of the GH values exceeded two times the minimal detectable concentration in patients 1 and 4, and 100% of nocturnal values exceeded twice the detection limit in patient 3). Of interest, the four patients we studied varied over a threefold range in their GH production and peak amplitude despite the fact that they carried the same genetic defect. However, there was no correlation between GH production and stature or serum IGF-I level. Other parameters of GH pulsatility, such as pulse frequency, half-duration of peak width, and plasma half-life, were similar to those in normal subjects (28, 71). ApEn analysis confirmed that GH dynamics in these patients are both real and nonrandom and that there is a greater degree of process disorder than in normal men. Our findings are in substantial agreement with another report on GH secretion in two patients with a different inactivating mutation in the GHRH-R gene (58).

The possibility must be considered that residual GH secretion was due to incomplete resistance to GHRH, either because low levels of GHRH-Rs were present or through interaction of GHRH with other, related receptors [e.g., vasoactive intestinal peptide (VIP) and/or pituitary adenyl cyclase-activating polypeptide (PACAP) receptors]. Theoretically, GHRH-R expression, despite the mutation, could result from an alternative translational start site downstream from the mutation or from low-level readthrough through the stop codon. Scrutiny of the entire GHRH-R gene (Ref. 44 and GenBank accession no. AC005155) reveals no downstream start site capable of encoding a functional GHRH-R [this includes reported splice variants (57)]. Furthermore, on the basis of the strength of the mutant stop codon and its RNA context as a chain terminator, as well as information about expression levels of mutant proteins resulting from similar nonsense mutations in other human diseases, we consider it highly unlikely that readthrough occurs at a level sufficient to explain our findings. GHRH binds with low affinity to VIP/PACAP receptors (26, 40, 77), and activation of type I PACAP receptors occurs at GHRH concentrations of 10–100 nM, levels at least 100-fold higher than those needed for GHRH-R activation (21). Because the GHRH concentration in the hypophysial-portal blood of our patients is unknown, it is impossible to ascertain directly whether residual GHRH action through PACAP/VIP receptors is responsible for GH pulsatility. It is reasonable to postulate that, in GHRH-R deficiency, portal GHRH levels are increased because of a lack of GH and IGF-I feedback on hypothalamic GHRH neurons. However, hypothalamic GHRH expression in the little mouse is only about threefold above normal (19); hence, the magnitude of the postulated GHRH increase in portal blood would appear to be modest. Furthermore, Roelfsma et al. (58) found no GH response to a pharmacological dose of GHRH in two patients with GHRH-R deficiency, although those same patients were able to respond to GH-releasing peptide-2. In the aggregate, these observations do not support a significant role for GHRH in GH pulse generation in our patients. Thus, whereas we cannot formally exclude residual GHRH action mediated through alternate receptors, we consider this a remote possibility.

It is generally believed that, in humans, GH secretory spikes result primarily from GHRH pulses and that their amplitude, timing, and interpulse nadirs are modulated by somatostatin (see introductory section). Whether or not ghrelin plays a role in pulsatility is not yet known. Oscillations in somatostatin may also induce GH pulses such as those seen in the presence of constant, high levels of GHRH (72, 79) or GHS (32, 35).

Fig. 2. Plasma GH responses to hexarelin (2 μg/kg iv) in 4 patients with GHRH-R deficiency, as measured by sensitive chemiluminescence assay. Note that the scale on the ordinate extends only to 1.5 μg/l. The numbers next to the curves refer to patient numbers listed in Table 1.
The paramount importance of GHRH for GH pulses of normal amplitude is obvious from the present study, in agreement with previous studies with a GHRH antagonist. Administration of the competitive GHRH antagonist (N-Ac-Tyr₁,D-Arg₂)-GHRH-(1–29) largely suppressed spontaneous GH secretion as well as the response to provocative pharmacological stimuli (33, 34, 51). However, in those studies, GH secretion was not completely abolished, a finding that was attributed to incomplete pharmacological blockade. The present study shows that, in men, the fundamental GH rhythm persists in a miniaturized version even in the absence of a GHRH-R signal. Therefore, the incomplete abolition of GH secretion by GHRH antagonist treatment may in part be explained by this GHRH-independent GH rhythm. Ours is a more severe paradigm of GHRH resistance than is GHRH antagonist treatment of normal subjects because of its chronicity, somatotrope hypoplasia, and impact on GH synthesis. GHRH can therefore be viewed to play a dual role in GH secretion. First, it acts as a pacemaker for GH secretory episodes through its own pulsatile pattern in the pituitary portal system. This has been directly shown in sheep, where there is a high concordance between hypophysial-portal GHRH pulses and GH pulses in the peripheral circulation (8, 20). It appears likely that the same obtains in humans, although no direct information is available. Second, GHRH acts as an important amplifier of an underlying GH rhythm, as revealed in the present study. Among the three biological actions of GHRH, somatotrope proliferation (6), GH synthesis (3), and GH release (11), it is primarily the first and second that subserve this amplifier role.

The present study also indicates that factors other than GHRH contribute to the augmented nocturnal GH secretion. Both baseline and pulse amplitudes were higher at night than during the day in our patients, a phenomenon that clearly cannot be attributed to GHRH. This is in agreement with other, more circumstantial evidence that enhanced nocturnal GH secretion is not fully attributable to GHRH (36, 48, 62, 69) and supports the concept that decreased somatostatin tone contributes to enhanced nocturnal GH secretion (70). In the absence of GHRH action, it is either somatostatin withdrawal, ghrelin pulses, or another, unknown oscillator that must be responsible for residual nocturnal pulse generation. The magnitude of the GH response to hexarelin in our patients is comparable to the spontaneous pulse amplitudes observed; hence, ghrelin would be a theoretical possibility. However, in the absence of any information about the physiological role of ghrelin in GH secretion, somatostatin would appear the most likely candidate at present. Other neuropeptides to be considered here are PACAP, VIP, galanin, and perhaps other hypothalamic factors acting through their own receptors. PACAP can act as a GH secretagogue in the rat but appears to lack this property in humans (see Ref. 56 for review). Similarly, VIP does not stimulate GH release in normal humans (10, 38). Galanin affects GH secretion primarily at the hypothalamic level by acting on neurons to release GHRH (47); direct pituitary effects are modest and seen only at pharmacological levels (42, 43). Thus none of these neuropeptides appears to be a compelling candidate for driving residual GH dynamics in our patients.

Pulsatility of GH secretion has been observed, albeit at varying amplitudes, under most, if not all, experimental conditions, including infusions with GHRH (72, 79), GHS (27, 32, 35), and somatostatinergic agents (7, 13, 61) alone or in combination (61), GHRH antagonist treatment (34), and now, genetic GHRH resistance. Thus, pulsatility appears as a very robust feature of GH secretion that persists despite attempts to disrupt hypothalamic input. This phenomenon suggests either that there is redundancy among hypothalamic factors in driving pulsatility or that a fundamental secretory rhythm is inherent in the somatotrope. Indeed, both possibilities may be true. The coordinate GHRH-somatostatin rhythm in the rat is an example of the former. Rat somatotropes incubated in vitro without hypothalamic peptides secrete GH in an oscillatory manner, a pattern that is related to oscillating transmembrane calcium fluxes (30). The amplitude, but not the frequency, of these oscillations is modulated by GHRH and somatostatin (67). Furthermore, monkey hemipituitary explants in a perfusion chamber secrete GH in a pulsatile pattern (63). These are examples of episodic secretory activity intrinsic to the somatotrope. These in vitro oscillations occur with a higher frequency (2–13/min and 12–15/h, respectively) than what is observed in vivo. However, the relatively short observation times of these studies (5 and 75–150 min, respectively) preclude conclusions about the potential existence of longer periodicities. Thus, it is unclear how much intrinsic somatotrope pulsatility contributes to the residual GH pulse pattern observed in the present study.

In summary, we report that patients with genetic, complete GHRH resistance exhibit residual pulsatile GH secretion with a greatly diminished amplitude yet normal frequency. The diurnal profile is qualitatively similar to normal, with enhanced GH secretion at night, including a raised baseline and higher peaks. These findings confirm the critical need for GHRH as a principal pacemaker and amplifier of GH secretion but also illustrate that other factors contribute to the pulsatile and diurnal pattern of GH secretion in men. We speculate that lowered somatostatin tone and rhythmic withdrawal are likely responsible for the nocturnally enhanced GH secretion, but we cannot exclude a role for ghrelin or a similar endogenous GHS in pulse generation. The intrinsically oscillatory nature of GH secretion by somatotropes in the absence of hypothalamic input may also contribute to the observed pulsatility.

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