Generation of growth hormone pulsatility in women: evidence against somatostatin withdrawal as pulse initiator

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Dimaraki, Eleni V., Craig A. Jaffe, Roberta Demott-Friberg, Mary Russell-Aulet, Cyril Y. Bowers, Peter Marbach, and Ariel L. Barkan. Generation of growth hormone pulsatility in women: evidence against somatostatin withdrawal as pulse initiator. Am J Physiol Endocrinol Metab 280: E489–E495, 2001.—To test whether endogenous hypothalamic somatostatin (SRIH) fluctuations are playing a role in the generation of growth hormone (GH) pulses, continuous subcutaneous octreotide infusion (16 μg/h) was used to create constant supraphysiological somatostatinergic tone. Six healthy postmenopausal women (age 67 ± 3 yr, body mass index 24.7 ± 1.2 kg/m²) were studied during normal saline and octreotide infusion providing stable plasma octreotide levels of 2,567 ± 37 pg/ml. Blood samples were obtained every 10 min for 24 h, and plasma GH was measured with a sensitive chemiluminometric assay. Octreotide infusion suppressed 24-h mean GH by 84 ± 3% (P = 0.00026), GH pulse amplitude by 90 ± 3% (P = 0.00031), and trough GH by 54 ± 5% (P = 0.0012), whereas GH pulse frequency remained unchanged. The response of GH to GH-releasing hormone (GHRH) was not suppressed, and the GH response to GH-releasing peptide-6 (GHRP-6) was unaffected. We conclude that, in women, periodic declines in hypothalamic SRIH secretion are not the driving force of endogenous GH pulses, which are most likely due to episodic release of GHRH and/or the endogenous GHRP-like ligand.

GROWTH HORMONE (GH) is secreted in a pulsatile fashion in animals and in humans. The mechanism of generation of GH pulses is not known. It has been suggested that GH pulses are initiated by hypothalamic GH-releasing hormone (GHRH) discharges, by periodic hypothalamic somatostatin (SRIH) declines, or are the result of the interaction of hypothalamic GHRH and SRIH (9). Moreover, the endogenous GH-releasing peptide (GHRP)-like ligand could also contribute to the generation of GH pulsatility (19).

In vitro and in vivo studies in animals have shown that acute termination of somatotroph exposure to SRIH reliably results in acute GH release (5, 18, 23, 30, 33). Therefore, a decline in endogenous SRIH may serve as a potential driving force for the generation of GH pulses. In fact, in male rats, SRIH appears to play an important role in the regulation of GH pulsatility (26, 32), whereas its role in other species may be less crucial (7). Studies in sheep point to hypothalamic GHRH as the principal generator of GH pulses (8, 21, 34, 35).

In humans, studies with the use of a competitive GHRH antagonist showed that GH pulsatility is dependent on the presence of hypothalamic GHRH (24). These data, however, cannot determine whether periodic hypothalamic GHRH release is responsible for the initiation of GH pulses or whether GHRH has only a permissive role by allowing somatotrophs to respond to other stimuli. The role of SRIH withdrawal as pulse initiator was suggested by studies showing that, during continuous intravenous infusion of GHRH, which presumably overrode the endogenous hypothalamic GHRH pulses, GH pulses persisted (36, 39). Similarly, during continuous intravenous administration of SRIH to healthy young men, GH pulse frequency was suppressed but not completely abolished, suggesting that at least some of the GH pulses were not due to SRIH withdrawal (4). However, in none of these studies were plasma concentrations of GHRH or SRIH actually measured in a serial fashion. Because of technical imperfections of the infusions and the short half-life of both GHRH and SRIH, their plasma levels could have been fluctuating, thereby artificially creating GH pulses. Additionally, the plasma levels created by the infusion might not have been sufficiently high to obscure the pulsatility of the endogenous GHRH and SRIH.

In this study, we address the role of SRIH in the initiation of spontaneous GH pulses by use of continuous subcutaneous infusion of the long-acting SRIH analog octreotide. The long half-life of octreotide allows the creation of a constant supraphysiological somatostatinergic milieu that would eliminate the effects of...
any endogenous SRIH fluctuations. Persistence of GH pulses under such conditions would show that periodic declines of endogenous SRIH are not the principal cause of GH pulses.

Postmenopausal women were chosen as the initial model for our studies. In contrast to men, who have several high-amplitude GH pulses in the early morning hours and long apulsatile periods during the day, women have relatively uniform GH pulses that are distributed evenly throughout the day (15). Thus the pattern of GH secretion in women facilitates studies examining a potential effect on GH pulse occurrence. The postmenopausal status of our subjects assured uniformity of gonadal steroid milieu during the different periods of the study and among the subjects.

METHODS

Subjects. The study protocol was approved by the Institutional Review Board and the General Clinical Research Center (GCRC) of the University of Michigan. Written informed consent was obtained from all subjects before participation in any study procedures. Six healthy postmenopausal women with mean age of 67 ± 3 yr (mean ± SD), mean height of 164 ± 5 cm, mean weight of 66.4 ± 3.4 kg, and mean body mass index of 24.7 ± 1.2 kg/m² were studied. All women had unremarkable medical histories and physical examinations, and they were not receiving any medications that could influence GH secretion. Baseline hematological and biochemical tests were normal. Women who were on hormone replacement therapy with estrogen with or without progesterone were asked to discontinue these medications 3 wk before and for the duration of the study. The postmenopausal status of all subjects was confirmed by elevated serum follicle stimulating hormone levels.

Protocol. The study was performed at the GCRC of the University of Michigan. All women were admitted and studied twice, once with continuous subcutaneous infusion of octreotide at the rate of 16 μg/h and once with continuous subcutaneous infusion of normal saline (NS). On both occasions, the infusion rate was 0.032 ml/h. The studies were performed in random order, and there was an interval of at least 1 wk between the two admissions. During each study, subjects had scheduled meals at 0800, 1200, and 1800, except on day 3, when breakfast was omitted and lunch was served after the completion of the protocol. The lights were turned on at 0700 and off at 2300; napping was not allowed.

Subjects were admitted at 2000 on day 1. A heparinized intravenous cannula was inserted in a forearm vein for the purpose of blood drawing and intravenous injections. The subcutaneous infusion was administered via a MiniMed pump in the abdominal subcutaneous tissue. The subcutaneous infusion was started at 2200 on day 1 and was continued uninterrupted until 1300 on day 3. Blood samples were drawn for plasma GH every 10 min from 0600 on day 2 until 1330 on day 3 in five subjects and until 1200 in the sixth subject. On day 3 at 0600, an intravenous bolus of GHRH-44 (0.33 μg/kg; Bachem, Torrance, CA) was administered, and at 0800, an intravenous bolus of thyrotropin-releasing hormone (TRH, 50 μg; Ferring Pharmaceuticals, Tarrytown, NY) was given. Blood was drawn for plasma thyroid-stimulating hormone (TSH) every 20 min from 0800 until 1000. At 1000, an intravenous bolus of GHRP-6 (0.33 μg/kg; Peninsula, Belmont, CA) was given. Additional blood samples were obtained for plasma octreotide levels every 20 min from 2200 on day 2 until 0400 on day 3 during the octreotide infusion. In five subjects, blood samples for plasma octreotide were also obtained every 10 min for 30 min, after the octreotide infusion was discontinued.

Assays. Plasma GH was measured in duplicate by a chemiluminometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA) with assay sensitivity of 0.01 μg/l as previously described (16). All samples from each subject were measured in the same assay. TSH was measured in singletons by a chemiluminometric assay at the Ligand Laboratory of the University of Michigan by use of commercially available kits purchased from Chiron Diagnostics (East Walpole, MA). Plasma octreotide levels were measured at Novartis Pharma in duplicates by means of a specific radioimmunoassay with intra- and interassay coefficients of variation of <10% (22).

Data analysis. Analysis of GH pulsatility was performed by Cluster Program Version 6.00, with cluster size 2 × 2 and t-statistic of 3 and 2 for detecting significant increases and decreases in GH, respectively. The minimum absolute peak value was set at 0.03 μg/l to minimize the effect of assay variability (3, 37). The mean GH pulse amplitude was calculated for each subject during NS or octreotide infusion as the average of the amplitude (peak-nadir) of all pulses. The mean interpulse GH was calculated using Cluster-defined valley concentrations. The 24-h GH profiles were also analyzed by waveform-dependent deconvolution to estimate the frequency of GH inputs, defined as the estimated secretory episodes of GH and GH half-life (38). Twenty-four-hour mean GH was calculated as the average of all GH values over a 24-h period. Twenty-four-hour trough GH was determined as the average of the lowest 5% GH values in a 24-h period.

The GH response to GHRH or GHRP-6 is expressed as the amplitude of the response, defined as the difference between the baseline (time 0) and the maximum GH values and as the area under the curve (AUC) of GH vs. time, by use of the trapezoidal rule. The magnitude of the TSH response to TRH was calculated in the same fashion.

All comparisons between NS and octreotide treatment were made by two-tailed paired t-test, after logarithmic transformation of the data when appropriate. Statistical significance was assumed when the P value was ≤0.05. Data are presented as means ± SE.

RESULTS

Octreotide levels. During the subcutaneous octreotide infusion, constantly high plasma levels of octreotide were...
achieved (Fig. 1). The mean plasma octreotide level was 2,567 ± 85 pg/ml.

Events after cessation of the octreotide infusion. In five subjects plasma octreotide and GH levels were measured every 10 min for 30 min after discontinuation of the octreotide infusion (from 1300 to 1330). During this period, octreotide levels continued to be stable: 3,007 ± 62 pg/ml at 1300 and 2,801 ± 124 pg/ml at 1330 (P = 0.30 by ANOVA; Fig. 2). There was no rebound increase of GH during the 30 min after the abrupt cessation of the octreotide infusion (P = 0.40 by ANOVA).

Spontaneous GH pulsatility parameters. In all subjects, octreotide profoundly suppressed GH secretion. Figure 3 shows the 24-h GH profiles of the six subjects during NS and octreotide infusion. The discrete parameters of GH pulsatility during NS and octreotide infusions are summarized in Table 1. During the octreotide infusion, 24-h mean plasma GH was suppressed by 84 ± 3% (P = 0.00026). The mean GH pulse amplitude was suppressed by 90 ± 3% (P = 0.00031). The inter-pulse mean GH was suppressed by 73 ± 3% (P = 0.00030) and the 24-h trough GH by 54 ± 5% (P = 0.0012). Despite the marked suppression of GH secre-

<table>
<thead>
<tr>
<th>Parameters of GH pulsatility</th>
<th>NS</th>
<th>Octreotide</th>
<th>%Suppression</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-h Mean GH, μg/l</td>
<td>0.73 ± 0.26</td>
<td>0.14 ± 0.08</td>
<td>84 ± 3</td>
<td>0.00026</td>
</tr>
<tr>
<td>Mean GH pulse amplitude, μg/l</td>
<td>1.64 ± 0.64</td>
<td>0.22 ± 0.13</td>
<td>90 ± 3</td>
<td>0.00031</td>
</tr>
<tr>
<td>24-h Mean interpulse GH, μg/l</td>
<td>0.24 ± 0.09</td>
<td>0.068 ± 0.03</td>
<td>73 ± 3</td>
<td>0.00030</td>
</tr>
<tr>
<td>24-h Trough GH, μg/l</td>
<td>0.08 ± 0.03</td>
<td>0.04 ± 0.01</td>
<td>54 ± 5</td>
<td>0.0012</td>
</tr>
<tr>
<td>Pulse frequency, pulses/24 h</td>
<td>9.5 ± 0.5</td>
<td>7.6 ± 1.6</td>
<td>0.35</td>
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GH, growth hormone; NS, normal saline.
tion by octreotide, there was no difference between the number of GH pulses occurring during the NS and the octreotide infusions (9.5 ± 0.5 vs. 7.6 ± 1.6 pulses/24 h, P = 0.35).

Deconvolution analysis could not fit the 24-h GH profile during octreotide infusion in the subject with the lowest 24-h mean GH. Therefore, the GH half-life and the number of GH inputs could not be estimated for this particular profile. In the remaining five subjects, there was no difference in the number of GH inputs during NS and octreotide infusion (12.6 ± 1.4 vs. 16.2 ± 3.8 inputs/24 h, P = 0.47). Even when the data from the omitted subject were included (8 GH inputs during NS and assumed zero inputs during octreotide infusion) in the entire group of six subjects, there was still no significant difference between the number of GH inputs during NS and octreotide infusion (11.8 ± 1.4 vs. 13.5 ± 2.8 inputs/24 h, P = 0.47). The GH half-life was similar during NS and octreotide infusion (22.3 ± 1.6 vs. 19.7 ± 2.8 min, P = 0.47). The total GH input was lower during the octreotide infusion (P = 0.0012).

**Growth hormone response to GHRH and GHRP-6.**

The amplitude of GH response to GHRH was suppressed by the octreotide infusion in five of six subjects. When the data from all six subjects were analyzed, there was no statistically significant suppression of the amplitude of the GH response to GHRH during the octreotide infusion (overall suppression 34 ± 27%, P = 0.10; Fig. 4). When the data from the single subject who did not suppress her GH response to GHRH during the octreotide infusion were excluded, the overall suppression of the GH response to GHRH was 59 ± 13% (P = 0.035). The mean GH response to GHRP-6 was small, with mean amplitude of 1.12 ± 0.31 μg/l during the NS infusion. The GH response to GHRP-6 was not suppressed during the octreotide infusion (P = 0.50). Similar results were obtained by comparing the AUC of the GH responses to GHRH and GHRP-6.

**Response of TSH to TRH.**

The TSH response to TRH was suppressed by 57 ± 6% during the octreotide infusion (12.49 ± 1.08 vs. 5.31 ± 0.75 μU/ml, P = 0.0027; Fig. 5). The AUC of the TSH response was decreased by 63 ± 5% (1,309 ± 112 vs. 467 ± 62 μU·min·ml⁻¹, P = 0.0014).

**DISCUSSION**

We have shown that, during octreotide infusion providing constant supraphysiological somatostatinergic tone, the GH pulse frequency remained unchanged. These findings suggest that, at least in women, periodic declines in hypothalamic SRIH discharge may not be important for the generation of GH pulses.

The role of SRIH withdrawal in the generation of GH pulses had been initially suggested by studies in rats. In vivo immunoneutralization studies and direct portal blood sampling in male rats suggested that there is reciprocal, rhythmic, episodic release of GHRH and SRIH and that the regular GH pulses occur as a result of episodic GHRH secretion at the time of a SRIH nadir (26, 32). In contrast, in female rats, SRIH immunoneutralization studies suggested that SRIH is secreted in a continuous rather than an episodic pattern (25).

In other species, the role of SRIH in the regulation of GH pulsatility appears to be less important. In sheep, GHRH bursts coincided with GH pulses, but there was no temporal relationship between either SRIH and GH or SRIH and GHRH pulses (8, 34). Moreover, unlike in rats, in sheep, active or passive immunization against SRIH did not alter GH pulsatility (21, 35). Therefore, there is gender and species variability in the role of SRIH in the generation of GH pulses, and extrapolation from animals to humans should be done with caution.

The importance of GHRH in GH pulse generation in humans is unquestionable. Administration of GHRH antagonist almost completely abolishes nocturnal GH pulsatility in young men (24). However, it is unclear whether GHRH is secreted in discrete pulses that are responsible for the initiation of GH pulses or is secreted tonically, permitting the occurrence of GH pulses only at the time of SRIH secretory declines.

**Fig. 4.** GH response to GH releasing hormone (GHRH; A) and to GH releasing peptide-6 (GHRP-6; B) during NS and octreotide infusion. Data are presented as means ± SE of GH of all 6 subjects at each time point. Response of GH to GHRH was suppressed by 34 ± 27% (P = 0.10). Response of GH to GHRP-6 was not suppressed.
Somatostatin was first considered to be important in the generation of GH pulses in humans when it was shown that pulsatile GH secretion persisted during continuous intravenous infusion of GHRH (36, 39). Therefore, it was proposed that GH pulses occur as the result of intermittent withdrawal of the SRIH inhibition. However, it is unclear whether the plasma GHRH levels created by the intravenous infusion were either constant or significantly higher than the concentration of endogenous GHRH in the hypophysial portal blood. The observed GH pulses could be the result of GHRH fluctuations created by the intravenous infusion or the result of endogenous GHRH pulses superimposed on the exogenous GHRH levels.

On the basis of the results of deconvolution analysis, Hartman et al. (10) have also concluded that GH is secreted in volleys composed of multiple, discrete secretory bursts and have suggested that this resulted from high-frequency GHRH secretory events superimposed on low-frequency episodes of SRIH withdrawal. In anthesis to this hypothesis, SRIH withdrawal produced only a weak GH response in humans (12, 13). Importantly, the acute GH response to SRIH withdrawal cannot be equated with the endogenous GH pulse: the occurrence of the former cannot be modified by pretreatment with GHRH-antagonist, whereas the occurrence of the latter is reliably eliminated by GHRH blockade (13, 14, 24).

In a previous study, administration of a continuous intravenous SRIH infusion to healthy young men suppressed GH secretion without eliminating the occurrence of GH pulses (4). However, because SRIH has a very short half-life (28), fluctuations in the delivery of SRIH could be responsible for periodic GH release.

In this study, we avoided the fluctuations of somatostatinergic activity by administering the long-acting SRIH analog octreotide as a continuous subcutaneous infusion. Whereas the half-life of SRIH is only 1–3 min (28), the plasma half-life of octreotide is ~2 h (6), allowing us to achieve constant plasma octreotide levels averaging ~2,500 pg/ml. In rats and sheep, the SRIH concentrations in the hypophysial portal blood are at the range of 10–100 pg/ml (8, 26, 34). Based on an EC₅₀ of 0.19 nM for suppression of GH secretion by human pituitary cells (29), we can assume that the SRIH concentrations in the hypophysial portal blood in humans are similar. Because the potency of octreotide in terms of GH suppression is 20–40 times greater than that of endogenous SRIH (1, 22), the plasma octreotide levels of 2,500 pg/ml provide somatostatinergic activity at least 500 times higher than the expected levels of native SRIH in the pituitary-portal blood. Therefore, we were able to create a constant supraphysiological somatostatinergic milieu that would negate the effect of any endogenous SRIH fluctuations. Importantly, plasma octreotide levels did not decline, and there was no rebound increase in GH levels for 30 min after abruptly discontinuing the subcutaneous octreotide infusion. Therefore, potential technical problems causing brief interruptions of the octreotide infusion could not have resulted in episodic release of GH that would give the false impression of pulses.

As expected (25), octreotide infusion suppressed the 24-h mean GH, the 24-h trough GH concentration, and the interpulse GH levels. The efficacy of the octreotide infusion was further confirmed by the suppression of the TSH response to TRH stimulation by ~60% (40).

Despite the obvious effects of the constant supraphysiological somatostatinergic milieu, GH pulses continued to occur at the same frequency, leading to the conclusions that periodic SRIH declines are not required for the generation of GH pulses and that GH pulses are the result of factors other than periodic SRIH declines.

Somatostatin could affect GH pulsatility at the pituitary level by blocking the GH response to GHRH or at the hypothalamic level by suppressing the activity of GHRH neurons. However, the high octreotide levels would not only inhibit the GHRH effect at the pituitary level, but would likely also reach the hypothalamic GHRH neurons. In guinea pigs, peripherally administered labeled octreotide accumulated in the cerebrospinal fluid and the hypothalamus (2). Intravenous BIM-23014, a SRIH analog similar to octreotide, inhibited the secretion of GHRH in the hypophysial portal blood in sheep (20). Thus it is unlikely that fluctuations in SRIH activity at the level of the hypothalamus are responsible for episodic release of GHRH resulting in GH pulses. Our results support the role of GHRH as the principal GH pulse generator. The octreotide infusion did not completely eliminate the GH response to GHRH; therefore, the GH pulses observed during the infusion could be due to episodic release of hypothalamic GHRH. The more marked suppression of the spontaneous GH pulse amplitude implies a combined effect of octreotide in suppressing both pituitary response to endogenous GHRH and hypothalamic GHRH release.

Sassolas et al. (27) observed that, in healthy young men, GH secretion continued to be pulsatile during
overnight simultaneous continuous administration of BIM-23014 and GHRH. These results raise the possibility of the existence of a third factor, other than GHRH or SRIH, which could play the role of GH pulse generator. In our study, GHRP-6, a synthetic peptide that stimulates GH secretion through the GH secretagogue (GHS) receptor, promoted GH release during both the NS and the octreotide infusions. An endogenous GHS receptor ligand, ghrelin, was recently identified and isolated in rat and human tissues, including the arcuate nucleus (17). Although the physiological role of this newly discovered peptide is not yet known, it might potentially fulfill the role of the missing “third factor” in the regulation of GH secretion.

In summary, we have shown that GH pulses clearly persisted under constant supraphysiologic somatostatinergic tone, leading to the conclusion that SRIH declines are not responsible for the generation of endogenous GH pulses and that transient decrease in the somatostatinergic tone is not required for the occurrence of a GH pulse. We suggest that GH pulses are the result of SRIH-independent, episodic secretion of hypothalamic GHHRH and that the endogenous GHRP-like ligand could play the role of an additional generator of GH pulses. Because the regulatory mechanisms of GH pulsatility are sexually dimorphic in animals (31, 32) and in humans (11, 15), these conclusions can currently apply only to women. Whether a similar model is operable in men would require further studies.

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