Pulsatile and nocturnal growth hormone secretions in men do not require periodic declines of somatostatin

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THE MAINTENANCE of pulsatile and diurnal growth hormone (GH) secretion is controlled by several factors, including hypothalamic GH-releasing hormone (GHRH) and somatostatin (SRIH). In addition, the recently discovered gastric peptide ghrelin may play a role. The relative role of each of these factors in coordinating pulsatile GH secretion is not clear. (11).

In vitro and in vivo studies in animals have shown that SRIH withdrawal reliably results in rebound GH release, suggesting decline in endogenous SRIH secretion as the driving force of GH pulses (4, 20, 26, 36, 38). In rats of both sexes, hypothalamic GHRH is critically important for GH pulse generation (31, 37), but the role of SRIH in the regulation of GH pulsatility is clearly sexually dimorphic. In male rats, SRIH appears to be secreted episodically and to play an important role in the regulation of GH pulsatility (31, 37). In contrast, in female rats, SRIH appears to be secreted in a more continuous fashion and is unlikely to be important in the generation of GH pulses (30). In other species such as sheep, hypothalamic GHRH appears to be primarily responsible for the generation of GH pulses (8, 23, 39, 41).

In humans, endogenous GHRH is required for GH pulsatility (29). However, it is not clear whether periodic hypothalamic GHRH release is responsible for the initiation of GH pulses or whether GHRH is required for the action of other factors. Previous studies in healthy young men showed persistence of GH pulses during continuous intravenous GHRH infusions, suggesting that periodic declines of the somatostatinergic tone were responsible for the initiation of GH pulses (42, 45).

Previous experiments in men by use of repeated boluses of GHRH showed increased somatotroph sensitivity to GHRH during the early morning hours, coinciding with the spontaneous nocturnal augmentation of GH secretion, suggesting that nocturnal decline of the somatostatinergic tone is responsible for the augmentation of GH secretion (17).

Using a model of continuous subcutaneous infusion of the long-acting SRIH analog octreotide to create a constant supraphysiological somatostatinergic milieu, we have previously shown (7) that, in postmenopausal women, endogenous SRIH fluctuations are not required for the initiation of GH pulses. However, GH secretion in humans is sexually dimorphic (16), and male SRIH knockout mice exhibited feminization of the GH-regulated hepatic enzymes (21). Thus the applicability of our earlier findings in women to men is...
uncertain. It is conceivable that, similarly to rodents, SRIH might be more important in GH regulation in men than in women.

In the present study, we have used the model of continuous subcutaneous octreotide infusion to test the role of SRIH in the generation of GH pulses in men. At the same time, we have revisited the role of somatostatinergic tone in the phenomenon of the nocturnal augmentation of GH secretion in men.

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. Nine men with mean age 26 ± 6 yr (mean ± SD) and mean body mass index 23.3 ± 1.2 kg/m² participated in the study and were included in the analysis. All subjects 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.

Protocol. The study was performed at the GCRC of the University of Michigan. All men were admitted and studied twice, once with continuous subcutaneous infusion of octreotide at the rate of 8.4 μg/h and once with continuous subcutaneous infusion of normal saline (NS). On both occasions, the infusion rate was 0.042 ml/h. The studies were performed in random order, and there was an interval of ≥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 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. On day 3, at 0600, an intravenous bolus of 0.33 μg/kg GHRH-44 (Bachem, Torrance, CA) was administered, and at 0800, an intravenous bolus of 50 μg of thyrotropin-releasing hormone (TRH; 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 GH-releasing peptide (GHRP-2; Peninsula, Belmont, CA) was given. A dose of GHRP-2 of 0.1 μg/kg was given to the first subject studied, but it did not elicit a GH response. This subject was not included in the analysis of the results of GH response to GHRP-2. Subsequently, GHRP-2 was administered at a dose of 0.2 μg/kg.

Additional blood samples were obtained for plasma octreotide levels every 20 min from 2200 on day 2 until 0400 on day 3 in one subject and every 1 h from 0600 on day 2 until 0600 on day 3 in eight subjects during the octreotide infusion. Blood plasma octreotide levels were also obtained every 10 min for 30 min after the octreotide infusion was discontinued in all nine subjects.

Assays. Plasma GH was measured in duplicate by a chemiluminescent assay (Nichols Institute Diagnostics, San Juan Capistrano, CA) with assay sensitivity of 0.01 μg/l, as previously described (17). All samples from each subject were measured in the same assay. TSH was measured in singlicate by a chemilumino-metric assay with sensitivity 0.01 μU/ml at the Ligand Laboratory of the University of Michigan using commercially available kits purchased from Chiron Diagnostics (East Walpole, MA). Plasma octreotide levels were measured at Novartis Pharma in duplicates using a specific radioimmunoassay with intra- and interassay coefficients of variation of <10% (25). Data analysis. 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.

Analysis of GH pulsatility was performed by Cluster Program, version 6.00, with cluster size 2 × 2 and t-statistics of 3 and 2 for detecting significant increases and decreases, respectively, in GH. The minimum absolute peak value was set at 0.03 μg/l to minimize the effect of assay variability (2, 43). Only pulses with amplitude (peak-nadir) >0.02 μg/l were considered significant on the basis of our data that showed that pulses with amplitude <0.02 μg/l are indistinguishable from a normal day and sampling variability (6). For each subject, the number of GH pulses during NS infusion was corrected by deducting the low-amplitude pulses that would have been rendered insignificant (i.e., with amplitude <0.02 μg/l) if they had been suppressed by the average percent suppression of GH pulse amplitude during the octreotide infusion (2). 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 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, GH half-life, and GH basal, pulsatile, and total secretion (44).

The diurnal pattern of GH concentration during NS and octreotide infusions was analyzed using 2-h blocks by ANOVA with repeated measures and pairwise comparisons using Tukey adjustment.

The GH response to GHRH or GHRP-2 was expressed as the area under the curve (AUC) of GH vs. time, calculated by the trapezoidal rule. The magnitude of the TSH response to TRH was calculated in the same fashion. Data were logarithmically transformed.

All comparisons between NS and octreotide treatment were made by two-tailed paired t-test. All data sets were tested for normalcy, and where the distribution of the data was skewed it was normalized by logarithmic transformation. 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 764.51 ± 11.62 pg/ml. When plasma octreotide levels were analyzed in 6-h blocks, they were slightly but significantly higher during the 0600- to 1200-h block (P < 0.05) but were similar afterwards.

Events following cessation of octreotide infusion. GH levels were measured every 10 min for 30 min after the octreotide infusion was discontinued (from 1300 to 1330). During this period, octreotide levels continued to be stable, between 868 ± 66 pg/ml at 1300 and 872 ± 66 pg/ml at 1320 (P = 0.96 by ANOVA). At 1330 (802 ± 70 pg/ml), plasma octreotide levels were still higher.
than the average octreotide levels during the octreotide infusion ($P = 0.16$). There was no rebound increase of GH during the 30 min after the abrupt cessation of the octreotide infusion ($P = 0.63$ by ANOVA).

Spontaneous GH pulsatility parameters. In all subjects, octreotide suppressed GH secretion. Figure 2 shows the 24-h GH profiles of all nine 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 $52 \pm 13\%$ ($P = 0.016$); the mean GH pulse amplitude was suppressed by $47 \pm 12\%$ ($P = 0.012$); and the 24-h trough GH was suppressed by $39 \pm 12\%$ ($P = 0.030$). By cluster analysis, the average number of GH pulses during the NS infusion was $6.7 \pm 0.5/24$ h. After correction for the very low amplitude GH pulses, the number of GH pulses during NS infusion that would still be taken into account if all pulses were reduced by the average pulse amplitude suppression by octreotide was similar to the number of GH pulses during the octreotide infusion ($5.3 \pm 0.8$ vs. $5.0 \pm 0.7$, $P = 0.56$).

Deconvolution analysis showed that there was a marked decrease in the estimated basal ($30 \pm 13\%, P =...
0.023), pulsatile (58 ± 13%, P = 0.0084), and total (57 ± 13%, P = 0.0074) 24-h GH secretion during the octreotide infusion. The estimated GH half-life was similar during the two treatments. The number of GH-secretory inputs decreased during octreotide infusion by only 14 ± 6% (13.8 ± 0.95 vs. 11.8 ± 0.98 inputs/24 h, P = 0.043).

Diurnal rhythm of GH secretion. When the data from both NS and octreotide infusions were formally analyzed in 2-h blocks by ANOVA with repeated measures, GH concentrations were maximal between 2400 and 0200 (P < 0.05 compared with all other time blocks except for 0200–0400). The magnitude of the nocturnal augmentation of GH, expressed as the ratio of mean GH concentration during the 2-h period between 2400 and 0200 to the 24-h mean GH, was similar in the two treatments (P = 0.18) (Fig. 3).

GH response to GHRH and GHRP-2. The GH response to GHRH was suppressed by the octreotide infusion in seven of nine subjects (Fig. 4). There was a significant overall suppression of the GH response to GHRH by 38.3 ± 14.7% (285 ± 76 vs. 130 ± 24 µg·min⁻¹, P = 0.012).

The GH response to GHRP-2 was not suppressed during the octreotide infusion (895 ± 144 vs. 839 ± 251 µg·min⁻¹, P = 0.32).

Response of TSH to TRH. The TSH response to TRH was suppressed in all subjects by an average of 54.1 ± 3.2% (1,227 ± 236 vs. 535 ± 81 µU·min·ml⁻¹, P < 0.001).

**DISCUSSION**

Studies in animals suggest that there is both sex and species variability in the role of SRIH in the regulation of GH pulsatility (8, 21, 37, 41). In humans, it was suggested that periodic decline of SRIH is the principal GH pulse initiator by the persistence of pulsatile GH secretion during continuous intravenous infusion of GHRH (42, 45). However, there are significant limitations of these studies. Because plasma GHRH levels were not measured, it is conceivable that GHRH levels created by the intravenous infusion were not constant or were not higher than the hypophysial portal blood GHRH concentration. Moreover, whereas spontaneous GH pulses in men are suppressed by GHRH antagonist, the GH responses to exogenous SRIH withdrawal were not (13, 14, 29). This argues against a role of SRIH withdrawal in the generation of spontaneous GH pulses.

We have studied the role of SRIH in the generation of GH pulses by using a model of continuous infusion of...
the long-acting SRIH analog octreotide to create a constant supraphysiological somatostatinergic tone that would render insignificant any fluctuations in endogenous hypothalamic SRIH output. We have previously shown that, in women, GH pulsatility persisted during the octreotide infusion, thus demonstrating that GH pulses are not initiated by SRIH withdrawal (7). However, GH secretion regulation in humans is sexually dimorphic (16), and our earlier finding might not be reliably applied to men.

In this study, we used the same model of continuous octreotide infusion to investigate the regulation of GH pulsatility in young men. Because men have lower GH concentrations and lower-amplitude GH pulses during daytime, we used a lower dose of octreotide in an effort to prevent suppression of GH concentrations below the assay sensitivity. As in women, we were able to create high, stable levels of octreotide during the infusion, averaging ~750 pg/ml. Importantly, the degree of suppression of the TSH response to TRH was comparable with that in women, indicating that both infusion rates created a plateau of somatostatinergic activity (7, 46).

The actual concentration of SRIH in the hypophysial-portal blood in humans is not known, but in anesthetized rats and in conscious primates and sheep it is in the range of 10–100 pg/ml (8, 27, 31, 39). The EC50 (half-maximal concentration for GH suppression) of SRIH for suppression of GH secretion by human pituitary cells is 0.19 nM (35), which is comparable to the in vivo SRIH concentrations. Similarly, the dissociation constants for human and rat pituitary SRIH receptors are virtually identical at ~1 nM (32). Therefore, the portal blood SRIH concentration in humans are likely to be similar to those in animal models, and the octreotide infusion created concentrations ≥7–8 times higher than endogenous SRIH in absolute terms. Based again on the data from animals, the SRIH fluctuations in the portal blood are of a magnitude of 30–50 pg/ml, or ~40% of baseline values (27, 31). During the octreotide infusion, endogenous SRIH fluctuations of the same absolute magnitude would be in the range of only 6–7% of the somatostatinergic bioactivity. Thus, overall, any potential fluctuations in the endogenous pituitary-portal SRIH concentrations would be insignificant on the background of grossly supraphysiological somatostatinergic milieu. As observed in our study in women, octreotide levels remained high and stable, and there was no rebound GH increase for ≥30 min after discontinuing the infusion. Therefore, any potential technical problems leading to brief interruptions of the octreotide infusion would be an unlikely cause of the observed GH pulses.

Both the pulse amplitude and the trough GH levels were suppressed, but GH pulses persisted during the octreotide infusion. Although there were apparently fewer GH pulses detected by Cluster during the octreotide infusion, the difference in pulse frequency was fully explainable by the suppression of pulses of very low amplitude in the range that was indistinguishable from the assay and sampling variability (6). Therefore, not only did pulses continue to occur but pulse frequency was also essentially unchanged by the octreotide infusion. Similarly, the octreotide infusion suppressed trough GH levels in men in a similar manner to that observed in women in our previous study (7). These findings show that, in either sex, SRIH declines are not required for the occurrence of GH pulses.

The results of the deconvolution analysis were similar. Both pulsatile and basal GH secretions were suppressed, implying that, even during interpulse periods, endogenous somatostatinergic activity is not maximal. The decrease in pulsatile secretion was due primarily to the decreased mass of each secretory input rather than to the small decrease in the frequency of inputs. Overall, there was an average decrease of only two inputs per 24 h during the octreotide infusion, probably due to the suppression of very small GH inputs to the point that they could not be differentiated from the basal secretion.

Our results do not rule out the possibility that the somatostatinergic tone is lower at the time of GH pulses. Studies using a SRIH antagonist would allow indirect quantification of the somatostatinergic tone during GH pulses and in the interpulse periods (40). However, our data clearly demonstrate that changes in somatostatinergic tone at the level of the pituitary gland are not required for the secretion of GH in a pulsatile fashion.

Another possibility would be that SRIH controls the occurrence of GH pulses by suppressing the hypo

Fig. 4. Plasma GH response to intravenous GH-releasing hormone (GHRH; A) and intravenous GH-releasing peptide-2 (GHRP-2; B) during NS and octreotide infusions. Δ. Decrease in area under the curve GH as % of baseline values.
lamic release of GHRH (26). Currently, there is no model that would allow testing this hypothesis in humans. However, studies in animals have shown that peripherally administered octreotide crosses the blood-brain barrier and reaches the hypothalamus. In guinea pigs, labeled octreotide accumulated in the cerebrospinal fluid and the hypothalamus (1). In sheep, intravenous BIM-23014, a SRIH analog similar to octreotide, inhibited the secretion of GHRH into the hypophysial portal blood (22). Therefore, it is most likely that the fluctuations of SRIH at the hypothalamic level were also masked by the octreotide infusion.

What, therefore, is the principal GH pulse generator in humans? We have shown that GHRH is required for nocturnal GH pulsatility in young men (29). However, in humans, it is still unknown whether GH pulses are the direct result of episodic hypothalamic GHRH release or whether tonically secreted GHRH is required for the occurrence of GH pulses that are initiated by another factor. In support of the latter hypothesis, in patients with inactivating mutations of the GHRH receptor there are detectable GH pulses, albeit of very low amplitude (24, 33). The recently discovered peptide ghrelin might play a role in GH pulse generation (19). The observation that in patients with GHRH receptor mutations there is a small but significant response to exogenous GHRP-2 would suggest that ghrelin could be the primary GH pulse initiator in this group of patients (12). The potential role of ghrelin as a GH pulse promoter is also supported by our observation that the GH response to GHRP-2 was not suppressed by octreotide. However, it is unlikely that ghrelin is solely responsible for the occurrence of GH pulses, because at the same time there was a marked suppression of GH pulse amplitude and GH pulsatile secretion. The lack of inhibition of the GH response to GHRP-2 by octreotide is in agreement with previous studies that showed a decreased effectiveness of SRIH or octreotide in suppressing GH response to GH secretagogue (GHS) compared with GHRH, possibly a manifestation of the proposed functional antagonism of SRIH by GHS (5, 9). It is conceivable that the prior administration of GHRH increases GH responsiveness to GHRP-2 by either upregulating GHS receptors or downregulating SRIH receptors. In addition, decline in serum IGF-I levels during the octreotide infusion might have blunted the inhibitory effects of octreotide on GH secretion. The GH response to GHRP is more sensitive to inhibition by IGF-I than the response to GHRH (10). It is therefore likely that the inhibition of GH response to GHRP-2 by the octreotide infusion is negated by the decrease in circulating IGF-I.

In contrast, the response of GH to GHRH was suppressed in the majority of subjects. Therefore, GHRH is a more likely candidate for the role of GH pulse initiator. Recently, Katakami et al. (18) showed that, in male rats, immunoneutralization of GHRH completely inhibited endogenous pulsatility and the GH responses to ghrelin, whereas immunoneutralization of ghrelin abolished gh responses to ghrelin while leaving endogenous GH pulsatility intact. This is powerful evidence in favor of the crucial role of GHRH and the unlikely role of ghrelin as GH pulse promoter. The suppression of the GH response to GHRH by octreotide was of lesser magnitude than the suppression of the amplitude of spontaneous GH pulses. We speculate that the suppression of the endogenous GH secretion was the result of suppression of hypothalamic release of GHRH by octreotide in addition to decreased pituitary responsiveness to GHRH. The recent finding of SRIH-induced suppression of ghrelin secretion supports the hypothesis that inhibition of ghrelin secretion by octreotide might have played a role in GH suppression as well (3, 28).

The genesis of the nocturnal augmentation of GH in humans is equally enigmatic. The responsiveness of GH to boluses of GHRH was maximal at the time of day that corresponded to the spontaneous nocturnal augmentation of GH (17). This prompted us to suggest the existence of a diurnal SRIH rhythm that was responsible for the increased pituitary responsiveness to GHRH at night. However, in the present study, the nocturnal augmentation of GH secretion persisted during the octreotide infusions and was of the same relative magnitude, indicating that non-SRIH-related factors affecting pituitary responsiveness to GHRH are responsible for the nocturnal augmentation of GH. The recent finding of a diurnal rhythm of ghrelin concentration in temporal concordance with the GH rhythm offers an exciting possibility of a hypothalamic-gastric system of GH regulation (34). It would also explain the suppression of nocturnal GH secretion by GHRH antagonist (29) as well as our earlier finding of blunting of the nocturnal augmentation of GH secretion during continuous infusion of GHRP, possibly as a result of homologous desensitization of ghrelin receptors (15).

In summary, we have shown that, in healthy men, the pulsatile secretion and the diurnal rhythm of GH persist in the presence of constant supraphysiological somatostatinergic tone, leading to the conclusion that SRIH declines in the pituitary-portal blood are not required for the occurrence of GH pulses or for the nocturnal augmentation of GH. Our data demonstrate that, in humans and irrespective of sex, SRIH is unlikely to play a major role in the control of GH pulsatility.

We propose a model in which GH pulses are the result of SRIH-independent episodic hypothalamic GHRH secretion and in which the diurnal rhythm of GH secretion, and in particular the nocturnal augmentation of GH, may involve periodic gastric ghrelin secretion.

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