Somatostatin withdrawal alone is an ineffective generator of pulsatile growth hormone release in man

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To assess the relative roles of growth hormone-releasing hormone (GHRH) pulse and somatostatin withdrawal as potential generators of pulsatile growth hormone (GH) release in humans, we studied GH responses to iv bolus GHRH (1 µg/kg) and to termination of a 4 h iv somatostatin infusion (7.2 µg·kg⁻¹·h⁻¹) in five normal young men, and in five men with previously diagnosed isolated GH deficiency. The patients were diagnosed 8–15 years previously on the basis of typical auxological and hormonal criteria, were treated with exogenous GH and were off GH therapy for 1.5–8.9 years prior to this study. Growth hormone rises to a bolus GHRH were similar between the controls and the patients (maximum GH 27.3 ± 15.3 vs 8.0 ± 4.0 µg/l). The controls exhibited only a small GH rise to somatostatin withdrawal (maximum GH 2.9 ± 1.2 µg/l), while the patients did not (maximum GH 0.7 ± 0.1 µg/l; p < 0.05). We conclude that somatostatin withdrawal by itself is an ineffective promoter of GH pulsatility. Periodic quiescence of somatostatinergic neurons must be associated with a concomitant GHRH pulse in order to result in a robust GH pulse.

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Pulsatile secretion of growth hormone (GH) is subject to stimulatory and inhibitory regulation by hypothalamic GH-releasing hormone (GHRH) and somatostatin (SRIH), respectively. Growth hormone-releasing hormone stimulates GH synthesis and release, while SRIH inhibits GH release without affecting its synthesis (1, 2). In rats and sheep, peripheral GH pulses closely followed hypothalamic GHRH pulses (3, 4) and were abolished by anti-GHRH immunoglobulin (3) or GHRH antagonist (5). In contrast, administration of anti-SRIH immunoglobulin resulted in elevated interpulse GH concentration but did not change GH pulse occurrence (3). These observations suggest that GH pulsatile release is stimulated by periodic discharges of GHRH, while low interpulse GH levels are maintained by SRIH tone.

Somatostatin may also have a role in generating pulsatile GH release. The cessation of SRIH infusion either in rats (6, 7) or in humans (8) produced a pulse of GH. The persistence of pulsatile GH secretion in humans during continuous GHRH infusion has been used as an argument for the role of SRIH withdrawal in the generation of GH pulsatile release (9). This may result either from a direct pituitary disinhibition of GH release (10) or from a hypothalamic disinhibition of GHRH release. Administration of antiserum to GHRH prior to the termination of SRIH infusion in rats prevented a GH rise (6, 7), suggesting that SRIH withdrawal elicited a GH pulse by stimulating hypothalamic GHRH release.

This hypothesis is supported further by observations that, in vivo, episodic reduction of pituitary portal SRIH occurred shortly before the rise in portal GHRH (3), that in vitro depletion of hypothalamic SRIH increased GHRH release (11) and that SRIH inhibited the release of hypothalamic GHRH (12). Taken together, these results support the possibility that in animals SRIH withdrawal may release GH by disinhibiting GHRH neurons. However, whether SRIH withdrawal by itself can produce GH secretion in the absence of GHRH in humans remains unknown.

This study was aimed at investigating the question of whether in vivo SRIH withdrawal alone can generate a GH pulse in the absence of GHRH in humans. To this end we have studied adult subjects with isolated GH deficiency (IGHD), as current knowledge on this condition suggests that the majority of these patients are deficient in GHRH (13). Plasma GH responses to an iv bolus of GHRH and to cessation of SRIH infusion in this group of subjects were compared to those in normal controls.

Methods

Subjects

The study group consisted of five male subjects (20–25 years) who had been diagnosed previously as having IGHD. Besides IGHD, all subjects were healthy and were
taking no medication. They were recruited from the cohort of patients who were diagnosed and followed in the past by the Pediatric Endocrinology Clinic at the University of Michigan. All IGHD subjects had been diagnosed in childhood by conventional criteria (14) and selected for the study because their auxological and hormonal data at diagnosis suggested severe or complete GH deficiency (Table 1). At the time of diagnosis, all five had delayed bone age, a height of less than the 5th percentile and a linear growth rate of < 3 cm/year. In all five, spontaneous GH secretion was low and maximum GH responses to insulin (0.1 U/kg iv) and arginine (0.5 g/kg iv) were below 6 μg/l. The four patients who had plasma insulin-like growth factor I (IGF-I) measured when this assay was available had subnormal levels. All five were treated with exogenous GH and showed a favourable growth response. Growth hormone treatment had been ceased when the patients reached their final height 1.5–8.9 years prior to this study. At the time of the study, their mean height was 166.8 ± 2.5 cm with a mean height Z-score of −1.6 ± 0.4. One subject (no. 4) had a final stature of 175 cm (Z-score −0.3), while the rest were 160–168 cm tall (Z-score −1.3 to −2.6). All had normal weight (68 ± 4 kg) and body mass index (24.4 ± 1.1 kg/m²). Five healthy young (22–29 years) men (height 179.4 ± 1.5 cm, p < 0.001 vs patients; Z-score 0.4 ± 0.2, p < 0.001 vs patients; body mass index 23.5 ± 1.5 kg/m², p = NS) served as a control group. All IGHD and control subjects had normal routine serum biochemistry and hematological screen, thyroid function, serum prolactin and testosterone concentrations at the time of the study.

Protocol

The protocol was approved by the University of Michigan Human Studies Committee and written informed consent was obtained from every subject. Subjects were hospitalized at the Clinical Research Center at the University of Michigan. Spontaneous GH secretion was assessed by frequent (every 20 min) sampling from 20.00 h on day 0 to 08.00 h on day 1. to study GH pulsatility during its most active period. After that, on three consecutive mornings all subjects underwent the following studies. Day 1: 0.9% saline was given iv (10 ml/h) between 06.00 and 12.00 h. At 12.00 h the infusion was terminated and an iv bolus of 0.9% saline was given. Day 2: 0.9% saline was given iv (10 ml/h) between 06.00 and 12.00 h, at which time the infusion was terminated and 1 μg/kg GHRH (Bachem, CA) was given as an iv bolus. Day 3: 0.9% saline was given between 06.00 and 08.00 h, at which time it was substituted for an iv infusion of 7.2 μg·kg⁻¹·h⁻¹ SRIH (Sigma, St Louis, MO) between 08.00 and 12.00 h. The infusion was then stopped and an iv bolus of 0.9% saline was given. The timing of these studies was chosen to minimize the possible influence of spontaneous GH pulses. During all stages of the protocol, blood samples were drawn every 20 min from a contralateral arm between 06.00 and 14.00 h (2 h after termination of either saline or SRIH infusion). In four IGHD subjects, a bolus iv dose of 1 μg/kg GHRH was given 2 h after cessation of SRIH infusion and blood was sampled for another 2 h at 20-min intervals. Plasma IGF-I was measured in an 08.00 h sample on the first day of the protocol. Plasma SRIH was measured prior to the initiation of SRIH infusion, after 4 h of the infusion and 20 min after termination of SRIH infusion. The saline control. GHRH bolus and SRIH infusion experiments were carried out on days 1, 2 and 3, respectively, in all subjects, because a single iv bolus of GHRH was not expected to influence GH responses to subsequent stimuli given at least 24 h after it. The choice of dosages of GHRH (1 μg/kg iv bolus) and SRIH infusion (7.2 μg·kg⁻¹·h⁻¹) were based on the former being shown to produce a maximal GH response in normal subjects (16) and the latter being adequate to produce near-maximal suppression of GH secretion (17).

Assays

Plasma GH (18), IGF-I (19) and SRIH (20) were assayed by double-antibody RIAs. For GH, the detectability limit was 0.3 ± 0.04 μg/l, and the intra-assay coefficient of

Table 1. Clinical and hormonal characteristics of isolated growth hormone-deficient patients at diagnosisa.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>CA (years)</th>
<th>BA (years)</th>
<th>Ht (cm)</th>
<th>Height Z-score</th>
<th>GV (cm/year)</th>
<th>GH (μg/l)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Overnight</td>
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<td></td>
<td></td>
<td></td>
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<td>Peak</td>
<td>Insulin</td>
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<td>0.4</td>
</tr>
<tr>
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<td>137.5</td>
<td>-2.9</td>
<td>3.0</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>7.8</td>
<td>5.0</td>
<td>102.8</td>
<td>-4.5</td>
<td>3.0</td>
<td>ND</td>
</tr>
</tbody>
</table>

a CA: chronological age; BA: bone age; Ht: height (< 5th percentile in all); Z-score: calculated according to Hamill et al. (15); GV: growth velocity over the preceding year; GH: mean of 37 samples 20 min apart between 20.00 and 08.00, normal > 3.2 μg/l; IGF-I: insulin-like growth factor I measured by Nichols Laboratories, normal > 0.7 IU/l; ND: not done.
variation was below 10%. All samples from each subject were processed in duplicate in a single assay. Growth hormone concentrations below the value of detectability limit were assigned the detectability limit in the calculations.

Data analysis

Pulsatile GH secretion was assessed by Cluster analysis (21) of individual 12 h profiles. A power function fit of local variance, a $1 \times 1$ point cluster size and a $t$ statistic of 1 were used to minimize type I and type II errors in pulse detection with every 20-min sampling (22). Growth hormone pulses were detected as statistically significant increases followed by significant decreases. Detection of false positive pulses was minimized also by arbitrarily setting a minimal pulse amplitude of 1.0 $\mu$g/l (increase from preceding nadir to largest value in the pulse). Data were compared using a paired or unpaired $t$-test or non-parametric analysis, as appropriate. Statistical significance was assessed at $p<0.05$. Results were expressed as means $\pm$ SEM.

Results

The mean plasma IGF-I concentration was lower in the IGHD than in the control subjects at the time of the study ($109 \pm 18$ vs $164 \pm 21$ $\mu$g/l, respectively; $p<0.05$). In normal controls, the plasma IGF-I varied between 97 and 216 $\mu$g/l. In two IGHD subjects (nos. 1 and 2) plasma IGF-I concentrations were within the normal range (139 and 164 $\mu$g/l). Pulsatile GH patterns (overnight sampling) in all subjects are shown in Fig. 1. The mean plasma GH concentration and pulse frequency were similar between the two groups during the 12 h overnight sampling ($1.9 \pm 0.7$ and $1.8 \pm 0.5$ $\mu$g/l, 2.2 $\pm$ 0.9 and 1.4 $\pm$ 0.2 pulses/12 h in the IGHD and normal subjects, respectively). The mean pulse amplitude was lower in the IGHD than in the normal subjects (3.8 $\pm$ 1.5 and 7.6 $\pm$ 0.6 $\mu$g/l; $p<0.05$). The apparent discrepancy between the similar mean GH concentrations and GH pulse frequencies in both groups and the lower GH pulse amplitude in the patients suggests that some GH pulses of low amplitude might not have been identified as such (e.g. patient no. 5 at around 04.00 h) and/or the multicomponental GH secretory episodes (e.g. control nos. 2 and 4) were not resolved into separate discrete pulses. This was likely to be due to the relatively infrequent blood sampling and/or erratic performance of the algorithm in the low hormone range. The sensitivity of the RIA employed in this study was insufficient to describe accurately the basal interpulse GH concentrations. Thus, the contribution of this parameter to the total GH secretion is uncertain, and a valid comparison of interpulse GH between the two groups cannot be made. Spontaneous nocturnal GH pulses of 10–20 $\mu$g/l amplitude were observed in two patients with the previous diagnosis of IGHD. It should be noted that at diagnosis one of them (no. 1) had a spontaneous nocturnal GH pulse of 7 $\mu$g/l but very low (<2 $\mu$g/l) GH responses to provocative tests, while another one (no. 2) had a very low and virtually apulsatile GH secretion but a peak GH rise to arginine of 5.6 $\mu$g/l. Both had exceedingly low plasma IGF-I concentrations at diagnosis, despite being midpubertal.

During the saline infusion study, one control subject and one IGHD patient had a spontaneous GH pulse of around 08.00 h, but termination of saline infusion followed by saline bolus at 12.00 h was not followed by an increase of GH above the baseline in either group (Fig. 2). Administration of a GHRH bolus elicited prompt GH responses of similar ($p>0.1$) magnitude in both groups: 27.3 $\pm$ 15.3 and 8.0 $\pm$ 4.0 $\mu$g/l. Even though the difference was not significant statistically, the mean response in normals appeared to be threefold higher than in the patients. As one normal control had an unusually robust GH response to a maximum of 86.9 $\mu$g/l, this could have influenced the final result. Thus, we repeated the statistical comparison between the two groups using non-parametric analysis (Mann–Whitney test). Plasma GH increments to GHRH were 4.2–86.9 $\mu$g/l in controls (median 10.7 $\mu$g/l) and 2.0–23.3 $\mu$g/l (median 5.1 $\mu$g/l) in the patients ($p=0.39$).

Plasma SRIF concentrations in normal and IGHD subjects during SRIF infusion were as follows: basal $96 \pm 6$ and $112 \pm 19$ ng/l; 3790 $\pm$ 635 and 3413 $\pm$ 869 ng/l after 4 h of SRIF infusion; 260 $\pm$ 22 and 210 $\pm$ 22 ng/l 20 min after SRIF withdrawal ($p>0.1$ at all points between controls and IGHD subjects). Prior to the initiation of SRIF infusion, one patient (no. 1) had a spontaneous GH pulse to a maximum of 7.6 $\mu$g/l, but his subsequent response to SRIF withdrawal did not differ from the other patients. Termination of SRIF infusion (Fig. 2) in normal subjects was followed by a small but apparent rise in plasma GH to a maximum of $2.9 \pm 1.2$.


Fig. 2. Plasma growth hormone (GH) dynamics during control study (saline infusion/saline bolus), saline infusion and growth hormone-releasing hormone (GHRH) bolus and somatostatin (SRIH) infusion/saline bolus in control subjects (upper panel) and in patients with isolated GH deficiency (IGHD) (lower panel). Data are shown as means ± SEM: *p < 0.05 vs saline control; †p < 0.05 vs IGHD. Note different GH scales for different stages of the protocol.

μg/l, with the range of GH increments from nadir to peak (Δ GH) between 0.6 and 6.0 μg/l (2.6 ± 1.1 μg/l). This was significantly (p < 0.01) smaller than the spontaneous GH pulse amplitude in the same group. The SRIH withdrawal in IGHD subjects was virtually ineffective in promoting a rebound GH rise (maximum GH 0.7 ± 0.1 μg/l; p < 0.05 vs controls) with the range of Δ GH between 0 and 0.4 μg/l (0.2 ± 0.1 μg/l; p < 0.05 vs controls). To ascertain whether this lack of response was due to low releasable GH, another bolus of 1 μg/kg GHRH was given to four IGHD subjects 2 h after cessation of SRIH infusion: their maximal response to the first GHRH bolus (day 2) was 9.6 ± 4.7 μg/l; to SRIH withdrawal 0.7 ± 0.1 μg/l (p > 0.1 vs saline) and to the second GHRH bolus 14.1 ± 6.0 μg/l.

Discussion

To study whether SRIH withdrawal alone can produce a GH pulse, we conducted a study in men with previously-diagnosed isolated GH deficiency. This condition is believed to result from congenital absence of hypothalamic GHRH, because long-term administration of GHRH in these subjects restores GH secretion and promotes statural growth (23, 24). As the severity of GHRH deficiency in subjects with IGHD is variable (13) and our study required as complete a lack of spontaneous GHRH secretion as possible, we have selected individuals whose auxological and hormonal parameters at diagnosis were compatible with the most severe form of GHRH deficiency (14). Indeed, at diagnosis in all of them only minimal GH secretion was detected and GH responses to standard provocative stimuli were usually absent. Surprisingly, during adulthood, two of the five individuals exhibited apparently normal spontaneous GH secretion, as exemplified by the presence of large spontaneous nocturnal GH pulses and normal plasma IGF-I concentrations. It would be tempting, in retrospect, to reclassify these patients as originally having constitutional growth delay instead of classic IGHD. Whether their final short stature makes the misdiagnosis less likely and whether there is a continuum between the two conditions is uncertain. Clayton et al. (25) have shown that 25% of IGHD patients normalize GH responses to insulin, arginine or l-DOPA when tested 2–15 years after completion of GH therapy, and Cacciari et al. (26) have found that within 1–2 years of the initial diagnosis almost half of the IGHD patients exhibited normal spontaneous and/or pharmacologically stimulated GH secretion. Both groups exhibited apparently similar GH (and, by inference, GHRH) pulse frequency, and the mean spontaneous GH pulse amplitude was the only discrete parameter that was different between the patients and normal controls, although robust GH pulses were found in two patients with normal IGF-I concentrations. Together with the normal pituitary sensitivity to GHRH, this suggests that the amount of GHRH per pulse may still be abnormally low in IGHD patients, even during their adulthood.

Hindmarsh et al. (8) have shown that termination of SRIH infusion in normal men is followed by a rebound rise in plasma GH concentrations over the ensuing 2 h. Our data in normal controls are in complete agreement with their findings. However, in patients with previously diagnosed growth failure, termination of SRIH infusion was virtually ineffective in promoting GH rise. This is similar to an earlier observation by Peter and Szentistványi (27) of the rebound GH rise in normals to an iv bolus of SRIH and its absence in IGHD patients. The absence of GH rises to IGHD withdrawal in our subjects cannot be explained by the unavailability of the readily releasable GH, because a GHRH bolus given either before or shortly after termination of SRIH infusion elicited normal GH responses. Several lines of observation in animals suggest that the in vivo GH rebound to SRIH withdrawal may involve endogenous GHRH: a decline in the pituitary-portal SRIH concentrations preceded the rise in GHRH and GH pulse (3); abrupt withdrawal of SRIH infusion is more effective in vivo than in vitro (10); the rebound GH rise is blocked by GHRH antiserum (6, 28); and there are synaptic connections between SRIH and GHRH neurons (29). Whether a similar mechanism is operative in humans is uncertain, but the virtual absence of GH responses to SRIH withdrawal in patients...
with previously diagnosed abnormal GHRH secretion cautiously suggests that the rebound GH rises may at least partially involve endogenous GHRH.

In conclusion, we have shown that some patients with previously diagnosed IGHD may exhibit almost normal pulsatile GH secretion during adulthood. The termination of SRIH infusion cannot elicit the robust rebound GH rise either in normal controls or, especially, in the patients with previously diagnosed growth failure. Thus, periodic fluctuations of endogenous hypothalamic SRIH secretion are unlikely by themselves to be responsible for spontaneous GH pulsatility in man. A decline in SRIH tone must be associated with a concomitant GHRH pulse in order to be effective in promoting a robust GH pulse.

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References


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