Pulsatile Growth Hormone Secretion in Normal Man during a Continuous 24-Hour Infusion of Human Growth Hormone Releasing Factor (1-40)

Evidence for Intermittent Somatostatin Secretion

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Abstract

Growth hormone (GH) secretory patterns were studied in a patient with ectopic growth hormone releasing factor (GRF) secretion and in normal men given continuous infusions of human growth hormone releasing factor (1-40)-OH (hGRF-40). In the patient with ectopic GRF secretion, GH secretion was pulsatile despite continuously elevated immunoreactive GRF levels. To determine if pulsatile GH secretion is maintained in normal subjects, we administered to six healthy young men vehicle or hGRF-40, 2 ng/kg per min, for 24 h and gave a supramaximal intravenous bolus dose of hGRF-40, 3.3 μg/kg, after 23.5 h of infusion. hGRF-40 infusion resulted in greater GH secretion than did vehicle infusion and pulsatile GH secretion was maintained throughout hGRF-40 infusion. During the 23.5 h of vehicle infusion, total GH secretion (microgram; mean±SEM) was 634±151 compared with 1,576±284 during hGRF-40 infusion ($P = 0.042$). The GH response to the intravenous bolus of hGRF-40 was greater after vehicle infusion than after hGRF-40 infusion; 877±170 and 386±125 μg of GH was secreted after the bolus on vehicle and hGRF-40 days, respectively ($P = 0.015$). The total amount of GH secreted during the 25.5 h of the two study days was not different; 1,504±260 and 1,952±383 μg were secreted during vehicle and hGRF-40 days, respectively ($P = 0.36$). Not only was pulsatile GH secretion maintained during hGRF-40 infusion, but there was augmentation of naturally occurring GH pulses, which is in contrast to the effect of gonadotropin-releasing hormone on gonadotropin secretion. We suggest that GH pulses are a result of GRF secretion that is associated with a diminution or withdrawal of somatostatin secretion.

Introduction

In order to determine the effects of prolonged exposure of the somatotroph to growth hormone releasing factor (GRF),[1] we report a patient with acromegaly secondary to ectopic GRF production and the results of 24-h human growth hormone releasing factor (1-40)-OH (hGRF-40) infusions in normal men.

Synthetic hGRF-40 and hGRF-44 specifically stimulate growth hormone (GH) release by the anterior pituitary gland in normal men (1–3). During infusions of incremental doses of hGRF-40, 1, 3.3, 10, and 33 ng/kg per min, each for 90 min, there appears to be a dose-related increase of GH secretion during the 1–10-ng/kg per min infusions. During the 33-ng/kg per min dose, the effect wanes (4). However, there is great variability in the magnitude and timing of the GH responses among subjects. To determine whether this variability is related to the incremental manner of administration of hGRF-40, we performed a second series of studies in which normal subjects were given 6-h infusions of vehicle or hGRF-40, 1, 3.3, and 10 ng/kg per min on separate occasions (5). In these experiments, the maximal GH response was observed during the 3.3-ng/kg per min infusion, but there was great variability in the GH responses among and within subjects. In addition, 30 min before the end of the 6-h infusion each subject received a supramaximal bolus injection of 3.3 μg/kg of hGRF-40. The amount of GH released in response to the bolus was inversely proportional to the dose of hGRF-40 given during the infusion. These results suggested that, within the limitations of this experimental paradigm, GH secretion is pulsatile throughout 6 h of stimulation by hGRF-40 and a defined amount of GH is available for release. We now report our findings in a patient with ectopic GRF secretion and the nature of GH responsiveness in normal men during administration of hGRF-40, 2 ng/kg per min, continuously over 24 h with an additional intravenous bolus injection of 3.3 μg/kg after 23.5 h of infusion. The pattern of GRF and GH secretion in the patient with ectopic GRF secretion and our observations in normal men support the hypothesis that GH secretion is a result of the interaction of GRF and somatostatin, and indicate that the somatotroph does not become completely refractory to continuous stimulation by GRF.

Methods

Case report. W.K. is a 48-yr-old man with acromegaly secondary to ectopic production of GRF from a metastatic carcinoid tumor. He underwent a left lower lobectomy in 1963 for an asymptomatic lesion that proved to be a carcinoid tumor. Over the intervening 20 yr he had signs of progressive acromegaly (i.e., acral enlargement, excessive sweating, widening of teeth spaces). In 1982 he underwent a right total hip replacement for presumed degenerative joint disease. Microscopic examination of the femoral head revealed metastatic carcinoid cells. He was initially seen at the University of Virginia in 1983 for evaluation of acromegaly and possible ectopic GRF production. His

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1. *Abbreviations used in this paper:* GH, growth hormone; GnRH, gonadotropin-releasing hormone; GRF, growth hormone releasing factor; hGRF-40, human growth hormone releasing factor (1-40)-OH; LH, luteinizing hormone; rGRF, rat GRF.

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only pertinent medical history was a progressive increase in shoe and ring size and dental malocclusion. Physical examination was remarkable for a definite acromegalic appearance: normal blood pressure, dental braces, and minimal impairment of range of motion in the right hip.

Endocrine evaluation revealed normal levels of serum thyroxine (10.0 μg/dl; normal 4.5–11.5), thyroid-stimulating hormone (1.5 μU/ml; normal < 4), prolactin (11.7 ng/ml; normal < 20), testosterone (444 ng/ml; normal 300–1,000), luteinizing hormone (LH) (8.1 mIU/ml; normal 7.9–18.1), follicle-stimulating hormone (9.0 mIU/ml; normal 1.2–22.6), and morning plasma cortisol (10 μg/dl; normal 7–25). The serum somatomedin C was elevated at 5.2 U/ml (normal 0.62–1.79) as was the serum GH at 22 ng/ml (mean of 21 determinations through the day). A plasma immunoreactive GRF level was 11.3 ng/ml (normal < 600 pg/ml). After intravenous thyrotropin-releasing hormone, 500 μg, the serum GH rose from a baseline of 24 to a peak of 169 ng/ml. He was given intravenous regular insulin (0.3 U/kg), after which the plasma cortisol rose from a baseline of 9–19 μg/dl and the serum GH increased from 35.7 to 171.8 ng/ml. The serum GH values one-half hour and immediately before a 100-g oral glucose challenge were 25.4 and 11.5 ng/ml, respectively, and were 9.0, 21.8, 13.1, and 10.8 ng/ml at half-hourly intervals over 2 h. The increase in GH from a mean baseline of 18.5–21.8 ng/ml after glucose may represent a paradoxical response as described in another patient with ectopic GRF secretion (6), or may reflect spontaneous variation. Radiologic studies included a high resolution computerized tomography scan of the head, which revealed a pituitary gland of normal size with no evidence of tumor. A high resolution computerized tomography scan of the abdomen revealed a single low density lesion in the right lobe of the liver and a normal pancreas. A liver-spleen radionuclide scan also demonstrated a single lesion: an area of decreased uptake, 3 cm in diameter, in the superior portion of the liver. The patient was again studied in 1984, at which time blood samples for simultaneous determination of GH and immunoreactive GRF levels were obtained every 20 min during a 24-h period.

Experimental design—normal men. hGRF-40 was formulated as previously described (1, 2). The peptide solution was diluted with normal saline and administered in a dose of 2 ng/kg per min.

The studies were approved by the Food and Drug Administration and the Human Investigation Committees of the University of Virginia and the Salk Institute. Having given informed written consent, six healthy men, ages 20–32 yr (mean 26 yr), were studied. All subjects were within 15% of ideal body weight, had no medical problems, and took only ferrous sulfate between the study periods. Each subject was studied on two occasions which were separated by at least 30 d. They fasted from midnight until lunch, but were given water freely throughout the study. They were also given dinner at 1800 h and a snack at 2100 h. The subjects remained recumbent during the study, awake during the day, and were permitted to sleep through the night. Tobacco was not used during the studies. Blood pressure, pulse rate, and temperature were measured every hour from 0800 to 2200 h and from 0600 h the next morning until the end of the study at 1200 h. Blood samples for GH determinations were drawn every 15 min from 0800 h on the first study day until 1200 h the following day (total of 112 samples). Specimens for somatomedin C determinations were drawn at 0800 and 1800 h on the first day, at 0800 h the second day, and at 1000 h on the third day.

The protocol consisted of a control day (vehicle) and a hGRF-40 day in which normal saline was infused for the first 2 h (0800–1000 h), and was followed by a 24-h infusion of either vehicle or hGRF-40, 2 ng/kg per min. After 23.5 h of infusion, the subjects were given an intravenous bolus injection of hGRF-40, 3.3 μg/kg (0930 h, second day). From 1000 until 1200 h on the second day saline was again infused. The infusions were administered with a Harvard pump (Harvard Apparatus Co., Millis, MA). The total volume of infusate was 43 ml over 28 h.

Assays. Serum GH and somatomedin C were measured by standard radioimmunoassay (RIA) as described previously (7, 8). The sensitivity of the GH assay was 0.05 ng/tube. At 5 ng/ml the intraassay coefficient of variation was 11.5%. The intraassay variation for the somatomedin C assay was 5.8% at 0.3 U/ml and 6.6% at 1.22 U/ml. All samples from each subject were measured in the same assay to avoid interassay variation. Plasma GRF determinations were made by collection of 4½ ml of blood into plastic syringes that were kept at 4°C before blood drawing and the samples were immediately placed into heparin-containing tubes with 0.2 ml of aprotonin (Trasylol, 10,000 kallikrein inhibiting units/ml). The samples were immediately centrifuged at 4°C and the plasma removed and frozen. We have previously demonstrated that this method of collection prevents degradation of ^{125}I-hGRF-40 for at least 24 h at 4°C and at least 2 h at 37°C (9). In the absence of aprotonin, >10% degradation was noted at 6 and 1 h, respectively. The recoveries of exogenous hGRF-40 added to plasma treated with aprotonin were 99±6%. The plasma GRF RIA used unextracted plasma (10). Each plasma sample was assayed in at least two different dilutions, and in the present experiment, shown in Fig. 2, 10, 20, 50, 80, or 100 μl of plasma was added to each assay tube, and this was performed in duplicate. The volume of incubation was kept constant by varying the amount of buffer added.

hGRF-40 (synthesized as previously described [1]) was radioiodinated by the chloramine-T method as previously described (10). The intraassay coefficient of variation at a level of 1 ng/tube was 8.5%. The sensitivity of the assay was 120 pg/tube.

Methods of analysis. Growth hormone release was analyzed as integrated values for the time periods: 0800–1000 h (day 1, saline infusion), 1000–0930 h (day 1–day 2, continuous infusion period), and 0930–1200 h (day 2, after intravenous bolus injection of hGRF-40), and from 1000 h (day 1) through 1200 h (day 2). Integrated GH values per hour were calculated by computing the average serum GH level (nanogram per milliliter) for each measurement interval. The average values within a given time period were added (weighted by the length of the measurement interval) and then divided by the number of hours in the interval to obtain a per hour value. Integrated GH release (nanogram per milliliter per hour) on the control day was compared with integrated release on the hGRF-40 infusion day for the four time periods. Total GH secretion (microgram) during the various study times was calculated by multiplying the integrated GH secretion area (ng × min × ml^{-1}) by the GH metabolic clearance rate (190 ml × min^{-1}) (9, 12). Total GH and GRF secretion were also calculated in the same manner in the patient with ectopic GRF secretion; for GRF, the metabolic clearance rate used was 202 liter/m^2 per d (9). To determine if there was any significant relationship between serum GRF and GH levels in the patient with ectopic GRF secretion, regression analysis was performed by comparing simultaneous GRF and GH levels and by comparing GH levels with the GRF values of the preceding 20 and 40 min. All GH values of <0.5 ng/ml were considered equal to 0.5 ng/ml for the purpose of the calculations. Statistical significance was determined by analysis of variance as the time between treatments (i.e., >30 d) was sufficiently long to assume that each subject's responses on each study day were independent. Changes in serum somatomedin C levels were analyzed with the Wilcoxon rank sum test. Comparison of changes in somatomedin C levels before, 22 and 48 h after infusion between the vehicle and hGRF-40 days was made. Additionally, changes in somatomedin C levels after 8 and 22 h of infusion and 48 h after beginning the infusion (24 h after the intravenous bolus injection) on the same study day were compared.

Results

Acromegaly secondary to ectopic GRF production. In Fig. 1, both GH and immunoreactive GRF levels during a 24-h period are shown. GRF levels were in the 5–30 ng/ml range (mean 11.3 ng/ml; mean of 71 determinations) throughout the 24 h of the study. These levels are an order of magnitude above minimum levels of GRF necessary to stimulate GH secretion in normal subjects after infusion of hGRF-40 (9).

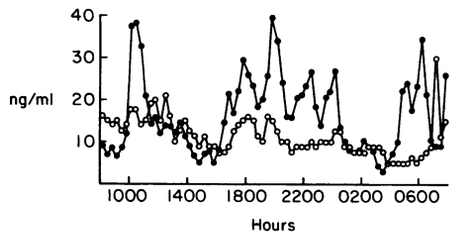

Figure 1. Serum GH (nanogram per milliliter) and plasma immunoreactive GRF (nanogram per milliliter) levels in a patient with acromegaly and ectopic GRF secretion. ●, GH; O, IR-GRF.

The pattern of GH secretion was pulsatile. Immunoreactive GRF levels varied and did not become undetectable during the 24 h of sampling. Regression analysis of simultaneous GRF and GH levels demonstrated an R^2 of 0.0634, $P = 0.035$. When GH values were compared with the preceding 20- and 40-min GRF levels, there was less correlation ($R^2 = 0.0401$, $P = 0.0990$; $R^2 = 0.0381$, $P = 0.1106$; respectively). The integrated GH secretion for the 24-h period was 15.8 ng/ml per h, and the total amount of GH secreted during the period was 4,256 μg. Integrated GRF secretion was 11.3 ng/ml per h and the total amount of GRF secreted was 4,000 μg. In Fig. 2, the displacement of ^{125}I-hGRF-40 by synthetic hGRF-40 and by different amounts of this patient's plasma is shown. Note that the displacement curves are not different.

Normal subjects. Spontaneous pulses of GH occurred in all subjects during the control (vehicle) day. These increases, which were more prominent during the night, are shown in Figs. 3 and 4. The GH levels between pulses were low (usually ≤0.5 ng/ml) on both vehicle and hGRF-40 days and the GH pulses were greater during hGRF-40 infusion than during vehicle infusion. Visual inspection of the data suggested a temporal relationship between the GH pulses on the vehicle and hGRF-40 days in that most of the increases occurred at the same times during both study days, particularly during the early hours of sleep. These patterns are suggestive of augmentation of naturally occurring GH surges by hGRF-40.

Review of individual subject responses to hGRF-40 and vehicle infusions (Fig. 3) reveals the pulsatile pattern of GH release on both study days and the spectrum of responsiveness

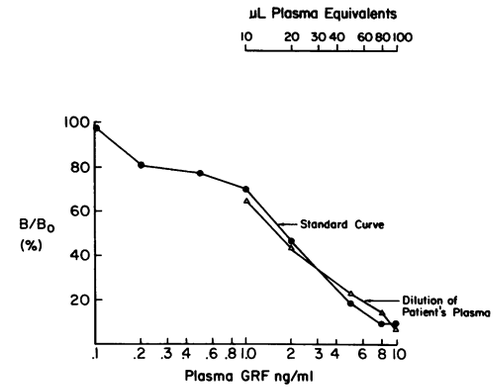

Figure 2. Displacement of ^{125}I-hGRF-40 by synthetic hGRF-40 in different amounts of plasma from the patient with ectopic GRF production. Note that the displacement curves are not different. ●, hGRF-40; △, W.K.

to hGRF-40 infusion. In subjects 2–4, the augmentation of naturally occurring GH peaks was obvious, while in subjects 1, 5, and 6 this was not evident. Additionally, GH secretion was unequivocally greater between 2000 and 0500 h in subjects 1–5, but not in subject 6.

GH release (nanogram per milliliter per hour; vehicle vs. hGRF-40 days; mean±SEM) during the initial 2 h of saline infusion was not different on the vehicle and hGRF-40 days (1.56±0.40 vs. 2.20±0.78; $P = 0.498$). The continuous infusion of hGRF-40, 2 ng/kg per min, resulted in a significant increase (2.37±0.56 vs. 5.88±1.06; $P = 0.015$) in GH release during the 23.5 h before the intravenous bolus injection. There was a marked increase in GH release during the 2.5 h after the supramaximal intravenous bolus dose of hGRF-40 on both study days, although on the hGRF-40 day it was only 44% of that observed on the vehicle day (30.77±5.95 vs. 13.54±4.38; $P = 0.042$). GH release during the combined periods of vehicle or hGRF-40 infusion and the 2.5 h after the bolus injection was not different between the two study days (5.07±0.88 vs. 6.59±1.29; $P = 0.36$).

When expressed as total GH secreted (microgram), there was no difference between the vehicle and hGRF-40 days during the initial 2 h of saline infusion ($P = 0.50$) (Fig. 5). Total GH secreted during the 23.5 h of hGRF-40 infusion was 2.5-fold greater than during the vehicle infusion ($P = 0.015$). Total GH secreted (mean±SEM) during the 2.5 h after the bolus injection of hGRF-40 was 877±170 μg on the vehicle day and 386±125 μg on the hGRF-40 day ($P = 0.042$, vehicle vs. hGRF-40 day). The quantity of GH secreted during the two study periods, i.e., continuous infusion and after intravenous bolus injection, was not different on the vehicle and hGRF-40 days ($P = 0.36$).

Changes in serum somatomedin C levels during (22 h) and 48 h after the infusion were not different between the vehicle and hGRF-40 days ($P > 0.05$). When changes in levels were compared with preinfusion levels on the same study day, there was a significant increase in somatomedin C levels only after 22 h of hGRF-40 infusion (Table I; $P = 0.035$). Somatomedin C levels were greater in five of five subjects 48 h after the hGRF-40 infusion (24 h after the intravenous bolus injection), but this did not reach the level of statistical significance and thus indicates only a trend ($P = 0.059$).

Discussion

Growth hormone secretion is regulated by the stimulatory peptide, growth hormone releasing factor, and the inhibitory peptide, somatostatin. The interaction of these peptides is likely a complex one and is not yet fully understood. GRF secreting tumors are associated with clinical acromegaly, thus suggesting that the somatotroph can be continuously stimulated to secrete excessive quantities of GH (6). However, the pattern of GH secretion in this disorder is not known, while in normal men GH secretion is pulsatile. Our studies were designed to observe the pattern of GH secretion in a patient with a GRF-secreting tumor and determine whether GH secretion was constant or pulsatile. In this patient we observed that his GH secretion was pulsatile. The pattern of GH secretion, while pulsatile, was abnormal in that there was no rise in GH during the period 2400–0500 h. This may represent an alteration of the normal circadian rhythm in this patient. This patient secreted approximately six times the amount of GH compared

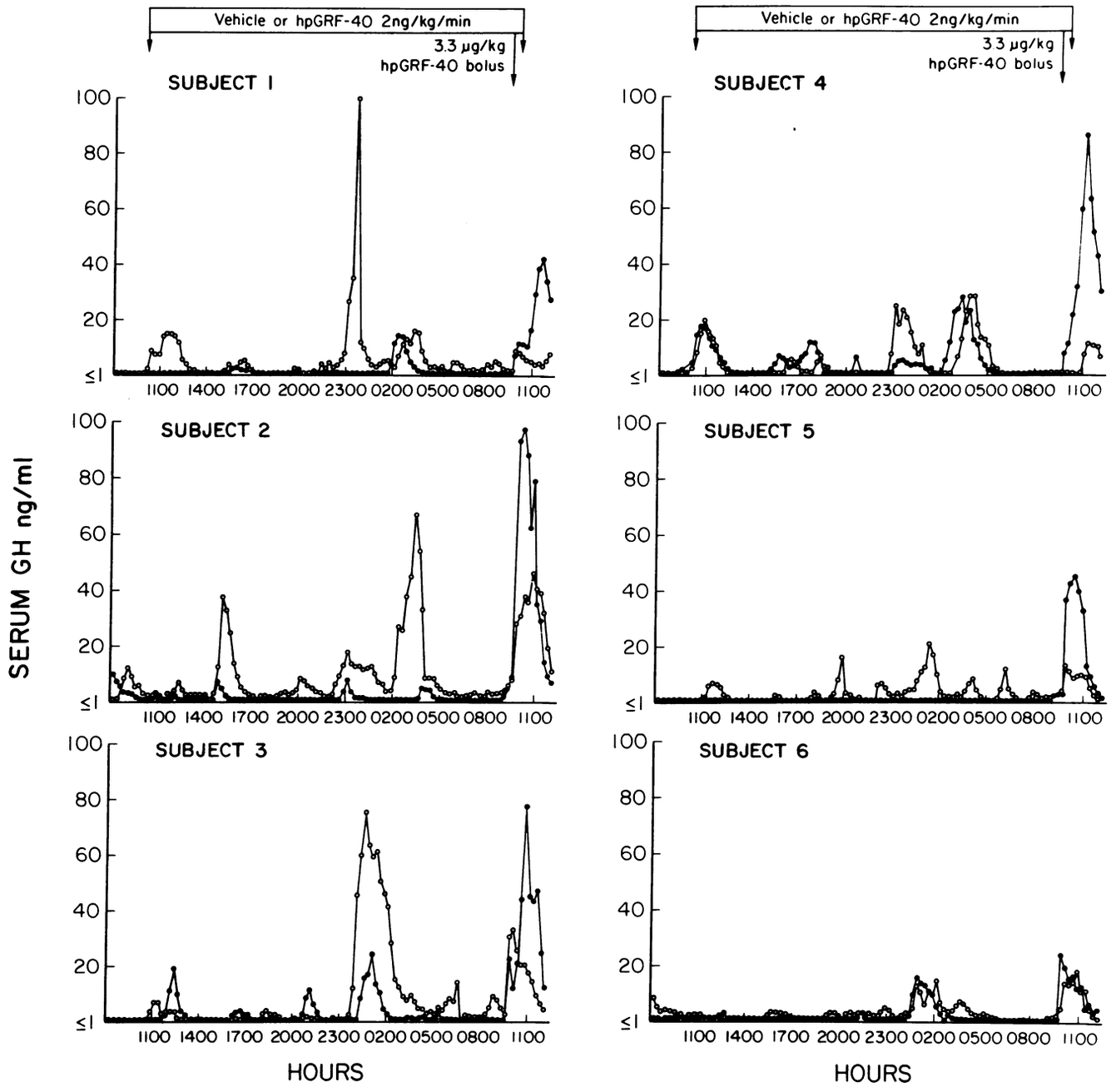

Figure 3. Serum GH (nanogram per milliliter) levels in six normal men given an infusion of vehicle (●) or hGRF-40 (○), 2 ng/kg per min, for 24 h, and hGRF-40, 3.3-μg/kg i.v. bolus, after 23.5 h of infusion.

to normal men under control conditions and two and one-half times that of normal men given hGRF-40 (i.e., ~4.2 mg vs. ~0.7 and 1.6 mg, respectively). We measured this patient's plasma immunoreactive GRF levels to determine whether the pulsatile pattern of GH secretion was influenced by changing circulating GRF levels, or alternatively, by varying pituitary responsiveness. Although the immunoreactive GRF levels did vary, they only accounted for 6% of the variation in GH secretion. In addition, the minimum levels observed (5 ng/ml) were 10–20-fold greater than the minimum levels we have observed that stimulate GH secretion in normal subjects (i.e., 300 pg/ml) (4). We calculated that this tumor secretes ~4 mg of GRF per day, and thus this patient was exposed to the equivalent of a continuous infusion of GRF at a dose in excess of 28 ng/kg per min.

We next examined the effects of a 24-h continuous infusion of hGRF-40 in normal young men. These studies demonstrated that the peptide stimulated GH release and augmented the naturally occurring pattern of GH secretion, particularly during the usual time of maximal GH secretion, i.e., during early morning hours of sleep. This pattern of GH secretion is different from that observed with gonadotropin secretion in subjects given gonadotropin-releasing hormone (GnRH) infusions. Infusion of GnRH results in biphasic release of LH and follicle-stimulating hormone with failure to return to base-line levels until apparent depletion or desensitization has occurred

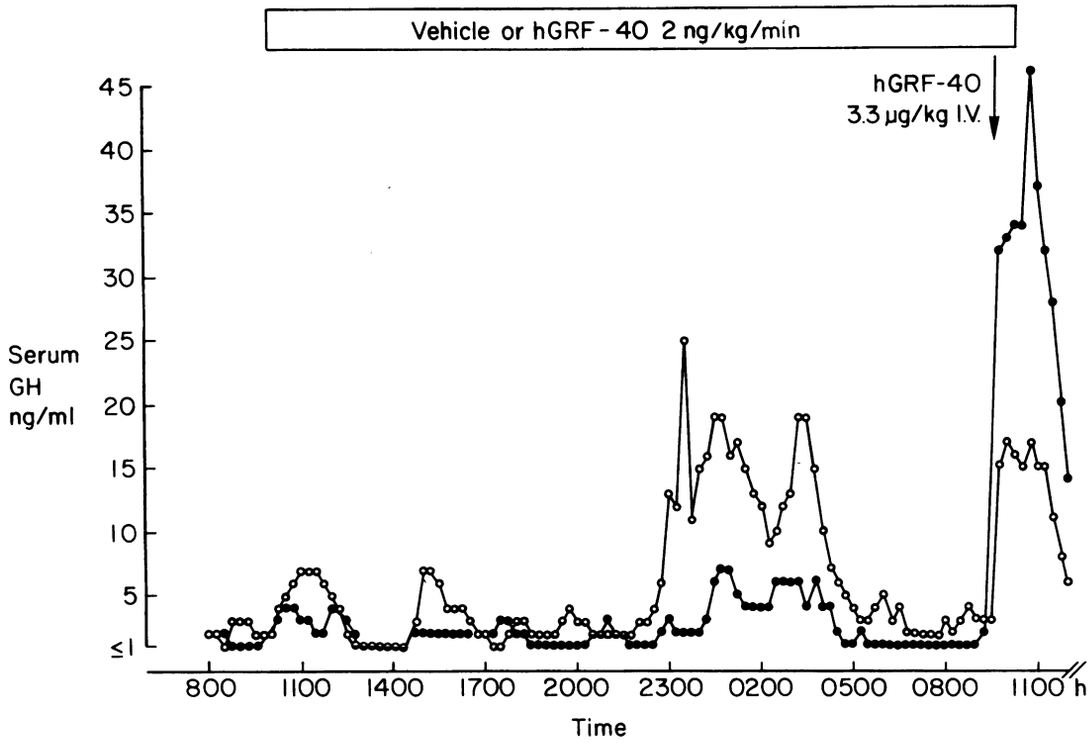

Figure 4. Mean serum GH (nanogram per milliliter) levels in 6 normal men described in Figure 1. Note the augmentation of GH secretion during hGRF-40 infusion, with greatest stimulation during the night. ●, Vehicle; ○, hGRF. $n = 6$.

(13–15). In contrast, during hGRF-40 infusion, the intrinsic GH rhythm is preserved, and GH levels intermittently fall to baseline during stimulation only to rise again as late as 23 h into the infusion. The preserved rhythmicity could be explained by pulsatile addition of endogenous hypothalamic GH-releasing hormone on a background of submaximal hGRF-40 levels produced by the infusion. We believe this is unlikely based upon our previous infusion studies in which we were able to demonstrate GH stimulation at a dose of 1.0 ng/kg per min (4). Similarly, the pattern of GH secretion observed in our patient with ectopic GRF secretion, in whom the GRF levels were extremely high throughout, would make this explanation unlikely. Rather, the preserved rhythmicity is more likely the result of the intermittent withdrawal of tonic inhibition of GH secretion by hypothalamic somatostatin. Peripheral somatostatin levels do not reflect changes in hypothalamohypophyseal portal concentrations. We have observed no changes in peripheral somatostatin levels after hGRF-40 administration (1, 2). These findings are consistent with the hypothesis that changes in hypothalamic and/or pituitary somatostatin concentrations are responsible for preservation of intermittent pulsatile GH secretion during hGRF-40 infusion. The elegant in vivo studies of Tannenbaum and Ling (16) in freely moving chronically cannulated rats are supportive of this hypothesis. These investigators demonstrated that GH responsiveness to both rat GRF (rGRF) and hGRF was dependent on the time of administration of the peptides; the GH responses to a single dose were three- to fivefold greater than baseline if the injection was given at 1100 h, which is the time of naturally occurring GH peaks. Injection of the peptide at 1300 h, a normal trough period of GH secretion, had no substantial effect on GH release. When animals were pretreated with antiserum to stomatostatin, a marked GH response occurred when GRF was given at 1300 h.

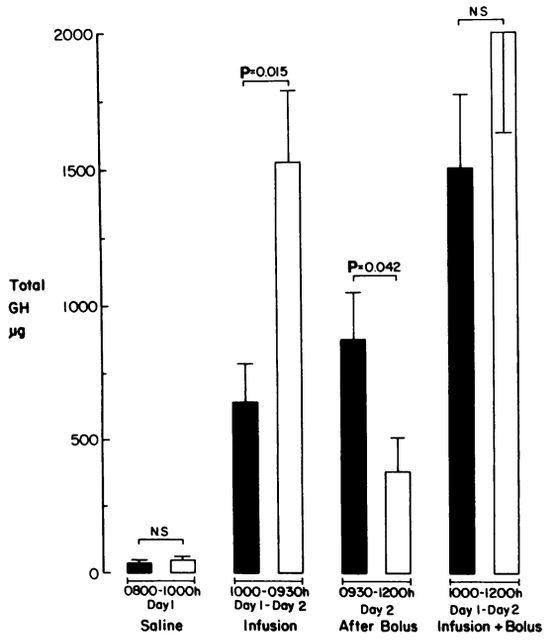

Figure 5. Mean±SEM total GH secretion (microgram) in six normal men: before (saline); during either vehicle or hGRF-40 (infusion); after i.v. bolus hGRF-40, 3.3 µg/kg (bolus); and during infusion plus bolus. ■, Vehicle; □, hGRF-40.

The observation of the reduced response to the supramaximal intravenous hGRF-40 dose observed after the 23.5-h infusion of hGRF-40 requires discussion. It is unlikely that pituitary GH was depleted by the hGRF-40 infusion, since the total amount of GH released over 26 h was 1.5±0.26 mg. The

*Table I. Serum Somatomedin C Levels**

	Vehicle			hGRF-40		
Subject	0800 (0 h)	0800 (22 h)	1000 (48 h)	0800 (0 h)	0800 (22 h)	1000 (48 h)
1	1.56	1.76 (+0.20)	1.48 (−0.08)	1.52	2.20 (+0.68)	4.04 (+2.52)
2	1.68	1.84 (+0.16)	1.64 (−0.04)	1.74	2.00 (+0.28)	2.08 (+0.36)
3	—	2.12 (—)	2.28 (—)	1.88	2.48 (+0.60)	2.80 (+0.92)
4	1.00	1.16 (+0.16)	1.20 (+0.20)	1.08	1.84 (+0.76)	1.36 (+0.28)
5	0.76	1.64 (+0.88)	1.45 (−0.12)	1.16	2.04 (+0.88)	— (—)
6	2.12	2.08 (−0.04)	— (—)	1.92	2.24 (+0.32)	2.20 (+0.28)
Mean±SEM	1.39±0.31	1.78±0.14 (+0.27±0.16)	1.61±0.18 (−0.01±0.07)	1.55±0.15	2.13±0.09‡ (+0.59±0.10)	2.45±0.45 (+0.87±0.43)

* Units per milliter. Levels shown before (0 h), during (22 h), and 48 h after beginning vehicle or hGRF-40 infusion in six normal men. Values in parentheses are change in somatomedin C as compared with 0800 h before infusion (0 h). ‡ $P = 0.035$ compared to 0 h, same day.

immunoreactive growth hormone content of the pituitary is 12.1±1.25 mg based on analysis of 15 individual human adult postmortem anterior pituitary glands preserved by freezing (Parlow, A. F., personal communication.) Similarly, the total amount of GH released over 24 h in the acromegalic patient was 4.3 mg. However, it is possible that a readily releasable pool of GH was depleted over time. This is supported by the finding of similar amounts of total GH released on the two study days in the normal men even though different total amounts of hGRF-40 were administered. There is some evidence from in vitro studies of rat anterior pituitary cells that depletion of cellular GH may occur. Bilezikjian and Vale (17) reported the effects of 24-h exposure of cultured cells to rGRF and the subsequent GH response to an additional 1 h of rGRF. They found that pretreatment of the cells for 24 h resulted in both depletion of cellular GH and diminished sensitivity to the subsequent rGRF stimulus; there was a 5.5-fold increase in the EC_{50}. The authors concluded that the attenuation of the response to the second rGRF stimulus was, in part, a result of depletion of GH. They also suggested that desensitization of the somatotroph to rGRF had occurred since 8 bromo-cAMP, which produced similar GH depletion, did not affect the EC_{50} of rGRF. The question of depletion or desensitization was also addressed by Foord et al. (18) in studies of cultured rat anterior pituitary cells. They compared the effects of hGRF-40 and GnRH on secretion of GH and LH from cells incubated for 24 h with increasing doses of hGRF-40 or GnRH and then given an additional 6 h of the stimulus. The amount of GH released during the 6-h exposure decreased with increasing doses of hGRF-40 used during the prior 24 h. However, the dose-response curves were identical when corrected for cell content, i.e., amount of GH available for release. There was no evidence for desensitization of the somatotroph. In contrast, the cells exposed to GnRH appeared to undergo marked desensitization. In summary, while in vitro data are controversial, it is possible that partial desensitization occurs leading to the reduced responsiveness observed in our studies in normal men.

Other mechanisms for this reduced response to the hGRF-40 bolus include negative feedback by other factors, such as GH and somatomedin C acting either directly at the pituitary or indirectly via the hypothalamus. Several investigators have shown that administration of GH to rats, either peripherally or intracerebroventricularly, results in a decrease in GH secretion (19, 20) and an increase in hypothalamic somatostatin concentration (21, 22). Moreover, endogenous somatostatin diminishes the GH response to GRF in rats, as demonstrated by the marked enhancement of the responses after neutralization of somatostatin by passive immunization (16, 23). In addition, preliminary studies in man demonstrate that 5 d of exogenous GH administration inhibits the subsequent GH response to an injection of GRF 12 h later (24). Thus, it is quite possible that GH feedback contributes to the reduced response of the supramaximal intravenous bolus injection, but this is likely mediated through somatostatin. Another candidate for negative feedback is somatomedin C. Administration of somatomedin C to unanesthetized rats suppresses GH secretion (20). In man, GH administration results in a rise in serum somatomedin C levels after 6 to 8 h (25). In our studies in normal subjects, small increases in serum somatomedin C levels after 22 h of hGRF-40 infusion were observed. Despite these increases, GH pulses occurred as late as 23.5 h into the infusion of hGRF-40. Additionally, the acromegalic patient had serum somatomedin C levels that were at least threefold elevated, and intermittent pulsatile GH secretion was preserved. These data suggest that somatomedin C is more likely to act to alter the set point for GH secretion instead of directly affecting pulsatile secretion, and is therefore unlikely to have contributed to the reduced response to the supramaximal dose of GRF administered at 23.5 h into the hGRF-40 infusion.

Based on our observations in the acromegalic patient with ectopic GRF secretion and the results of 24-h infusions of hGRF-40 in normal young men, we suggest that GH secretion in man is regulated by the dynamic interaction of GRF and somatostatin, and that, in the absence of blockade of the secretion of or effects of somatostatin, the somatotroph is capable of responding to prolonged GRF stimulation without becoming either depleted of total GH or completely refractory to this stimulus. These observations are not only of physiological importance, but may also be important in the design of therapy with GRF. In addition, our observations complement those of Tannenbaum and Ling (16) in the rat and offer support to the concept that pulsatile GH secretion in man is likely caused by a combination of enhanced GRF secretion with concomitant reduction of somatostatin secretion.

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