

# Plasma Growth Hormone Responses to Constant Infusions of Human Pancreatic Growth Hormone Releasing Factor Intermittent Secretion or Response Attenuation

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**A**bstract. Administration of human pancreatic tumor growth hormone (GH) releasing factor (hpGRF[1-40]) as a single injection to normal human subjects stimulates the secretion of GH in a dose-responsive manner. In the present studies, hpGRF(1-40) was infused in a graded stepwise manner over a 6-h period in order to determine whether the GH secretory response would be sustained. Normal adult males received four consecutive 90-min infusions of hpGRF(1-40) at doses of 1, 3.3, 10, and 33 ng/kg per min, preceded and followed by a 90-min saline infusion; and the plasma GH responses were compared with those during a separate control infusion.

Plasma GH levels were significantly elevated by each hpGRF(1-40) infusion; and dose responsiveness was evident for the lowest three doses. Mean integrated GH secretory rates for the four doses were 1.95, 3.29, 4.29, and 3.65 times those of the respective control study. Plasma GH responses exhibited considerable variability, frequently decreasing during the latter part of each infusion; and at the highest dose, they decreased continuously beginning shortly after the onset of infusion. Episodic GH secretion occurred in individual subjects during each of the infusion periods.

The possible contribution of hypothalamic somatostatin secretion to the diminished GH responsiveness was evaluated by determining plasma thyroid stimulating hormone (TSH) levels during the infusions and the TSH

responses to thyrotropin-releasing hormone (500  $\mu$ g i.v.) during a separate hpGRF(1-40) infusion of 2 ng/kg per min. Neither basal nor stimulated TSH levels differed between GRF-infused and control groups.

The results indicate that GH secretion is dose responsive to hpGRF(1-40) infusions, though the response is complex. The absence of impaired TSH secretion provides evidence against a mediating role of somatostatin. The explanation for the loss of GH responsiveness remains undetermined but could include GRF-induced receptor down-regulation, a postreceptor effect, or, in spite of our negative results, a somatostatin-mediated inhibition.

## Introduction

Growth hormone (GH)<sup>1</sup> secretion occurs in an episodic manner in response to a hypothalamic control mechanism, which involves a releasing and inhibiting factor. The regulation of GH secretion is complex and the interaction of the hypothalamic factors remains to be clarified. Structural characterization of a GH-releasing factor (GRF) that was isolated from ectopic production sites in two pancreatic tumors removed from patients with acromegaly has been recently reported (1, 2). A 40-amino acid peptide, human pancreatic GRF (hpGRF) (1-40), was the only GRF identified in one tumor (1) and the major component in the other tumor (2), which also contained hpGRF(1-44) and hpGRF(1-37). Although the structure of human hypothalamic GRF is not yet known, it appears to possess similar physicochemical and immunologic characteristics (3, 4); and two peptides that coelute with hpGRF(1-40) and hpGRF(1-44) have now been isolated and found to exhibit identical tryptic digest patterns and amino acid composition (5).

1. Abbreviations used in this paper: GH, growth hormone; GRF, GH-releasing factor; hpGRF, human pancreatic GRF; IR, immunoreactive; TRH, thyrotropin-releasing hormone; TSH, thyroid stimulating hormone; TFA, trifluoroacetic acid.

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Intravenous pulses of hpGRF(1-40) (1  $\mu$ g/kg) have been reported to stimulate GH secretion in normal men (6) and the responses are dose related, primarily during the second and third hour after injection (7). After the injection of higher doses of GRF, evidence of a biphasic GH response was observed, which raised the possibility of intermittent secretion or response attenuation. The present study was designed to explore this possibility by examining the GH secretory response to continuous stimulation by GRF by using a stepwise graded infusion. Since attenuation of the GH response could be mediated by increased hypothalamic somatostatin secretion (8, 9), we also examined basal and thyrotropin-releasing hormone (TRH)-stimulated thyroid stimulating hormone (TSH) secretion during GRF infusion to indirectly assess possible influences of somatostatin.

## Methods

### *Subjects studied*

Experiments were performed in 15 healthy adult males whose ages ranged from 22 to 30 yr and who were within 15% of ideal body weight. All subjects gave informed, written consent. Experiments were conducted under identical protocols at the General Clinical Research Centers at the University of Cincinnati and University of Virginia Hospitals to which subjects were admitted the evening before or the morning of study. The subjects consumed no food for 10 h before or during the study. Separate indwelling "butterfly" needles were placed in forearm veins and kept patent with heparinized saline for infusion/injection and blood sampling. Blood pressure and pulse were monitored throughout the study at frequent intervals. Subjects were studied in random order in each of the protocols.

### *Hormone preparation*

All experiments were performed with hpGRF(1-40), which was synthesized (1) and prepared (6) as previously described. Synthetic hpGRF(1-40) was diluted to a concentration of 100  $\mu$ g/ml with human serum albumin (1 mg/ml) and then further diluted in normal saline to provide an appropriate concentration for constant infusion rates as described below. All dilutions were made in plastic syringes. Blood pressure and pulse were monitored throughout the infusion.

### *Infusion protocols*

**Graded GRF infusion.** Eight subjects were infused for a 9-hour period beginning between 0800 and 0900 h. The study was divided into six 90-min segments. All subjects were infused with saline for the first 90 min. This was followed by sequential GRF infusions of 1, 3.3, 10, and 33 ng/kg per min, each of 90 min length. During the last 90 min, subjects were again infused with saline. The volume of infusate during the 9-h study ranged from 200 to 250 ml. On a separate day each subject was infused with saline for a 9-h period, thereby serving as his own control.

**GRF infusion-TRH injection.** Seven subjects participated in a three-infusion protocol, each of 5 h duration. In all protocols saline was infused during the 0-90-min and 210-300-min period. The three protocols for the 90-210-min period were as follows: In the first two protocols TRH was given 60 min after starting either saline infusion or GRF infusion (2 ng/kg per min). In the third protocol, saline was given as a bolus injection 60 min after starting a GRF infusion (2 ng/kg per min). The total volume of infusate during the 5-h study ranged from 100 to 130 ml.

### *Sample collection and processing*

Blood samples were collected in heparinized tubes containing sufficient aprotinin (Trasylol, FBA Pharmaceuticals, New York) to provide a final concentration of 1,000 kallikrein inactivator units/ml blood every 15 min during the studies and at 5-min intervals immediately after TRH injection. Samples were chilled, centrifuged, and stored at  $-70^{\circ}\text{C}$  until shipment to Cincinnati for subsequent studies.

### *GH and TSH radioimmunoassay (RIA)*

Plasma GH was measured by RIA as previously described (10). The assay sensitivity was 0.1 ng/ml and the intra- and inter-assay coefficients of variation were 6.5 and 7.8%, respectively. Plasma TSH was measured by RIA as previously described (11), which was modified by delayed addition of tracer and further dilution of anti-TSH serum to provide an assay sensitivity of 0.15  $\mu$ U/ml. The intra- and inter-assay coefficients of variation were 7.1 and 8.1%, respectively. In both radioimmunoassays, polyethylene glycol (PE 6000) was added 5-10 min after the second antibody to produce immediate precipitation.

### *GRF bioassay*

To test for possible adsorption to the plastic infusion syringes or degradation during infusion, the residual infusates from two of the graded GRF infusion protocols were appropriately diluted and bioassayed in primary monolayer cultures of rat pituitary cells (12, 13). Rat GH release into the incubation media was measured by RIA (14).

### *GRF RIA*

Plasma immunoreactive (IR)-GRF was measured by a double-antibody RIA using  $^{125}\text{I}$ -hpGRF(1-40) as tracer, rabbit anti-hpGRF(1-20), and hpGRF(1-40) as standard. The sensitivity of the assay was 15 pg/tube and the intra- and inter-assay coefficients of variation were 6.5 and 7.8%, at 1 ng/ml, respectively. IR-GRF was extracted from plasma by passage through a Sep-Pak (C-18 Cartridge, Waters Associates, Milford, MA), which was previously equilibrated with 0.01 M trifluoroacetic acid (TFA) and eluted with 80% acetonitrile in TFA. The details of the GRF RIA have been recently published (15). IR-GRF was undetectable in plasma samples obtained before infusion of hpGRF(1-40).

### *Statistical analysis*

Comparison of the GH and TSH secretory responses was made by an analysis of variance with repeated measures and by paired *t* test, as appropriate. Results were log-transformed before statistical analysis, when indicated, to improve comparability of variance. The *in vitro* GRF bioactive potency of the infusate was determined by a three-dose comparison with synthetic hpGRF(1-40) standard by means of covariance analysis.

## Results

**Plasma GH responses to graded GRF infusion.** Plasma GH responses to GRF and to saline infusions among individual subjects exhibited considerable variability. Although all plasma GH levels were  $<5$  ng/ml during the initial 90-min saline infusion in the 16 studies, all eight subjects demonstrated spontaneous pulses of GH secretion to levels of 5-36 ng/ml during the remainder of the control infusions. These pulses tended to occur during the latter portion of the study and were invariably noted during the last 90-min period. Secretory pulses of GH also occurred during the GRF infusion study, generally at times similar to those during the saline control infusions.

The results in four of the eight subjects are shown in Fig. 1 and are representative of the GH secretory patterns observed. Subject A exhibited a rise in GH levels in response to the lower three doses of GRF but not to the highest dose. A secretory pulse of GH also occurred after the end of the hpGRF(1-40) infusion. During the saline infusion, spontaneous GH secretion occurred at 2 and 8 h. In subject B, GH secretion increased in

response to the two lower doses of hpGRF(1-40) but not to the other doses. A postinfusion secretory pulse was also seen. However, the secretory pattern during the saline control infusion was qualitatively and quantitatively similar. The GH responses in subject C were similar to those of subject A, though a decrease in GH levels occurred toward the end of each of the infusion doses, during which a GH response occurred. GH secretion in

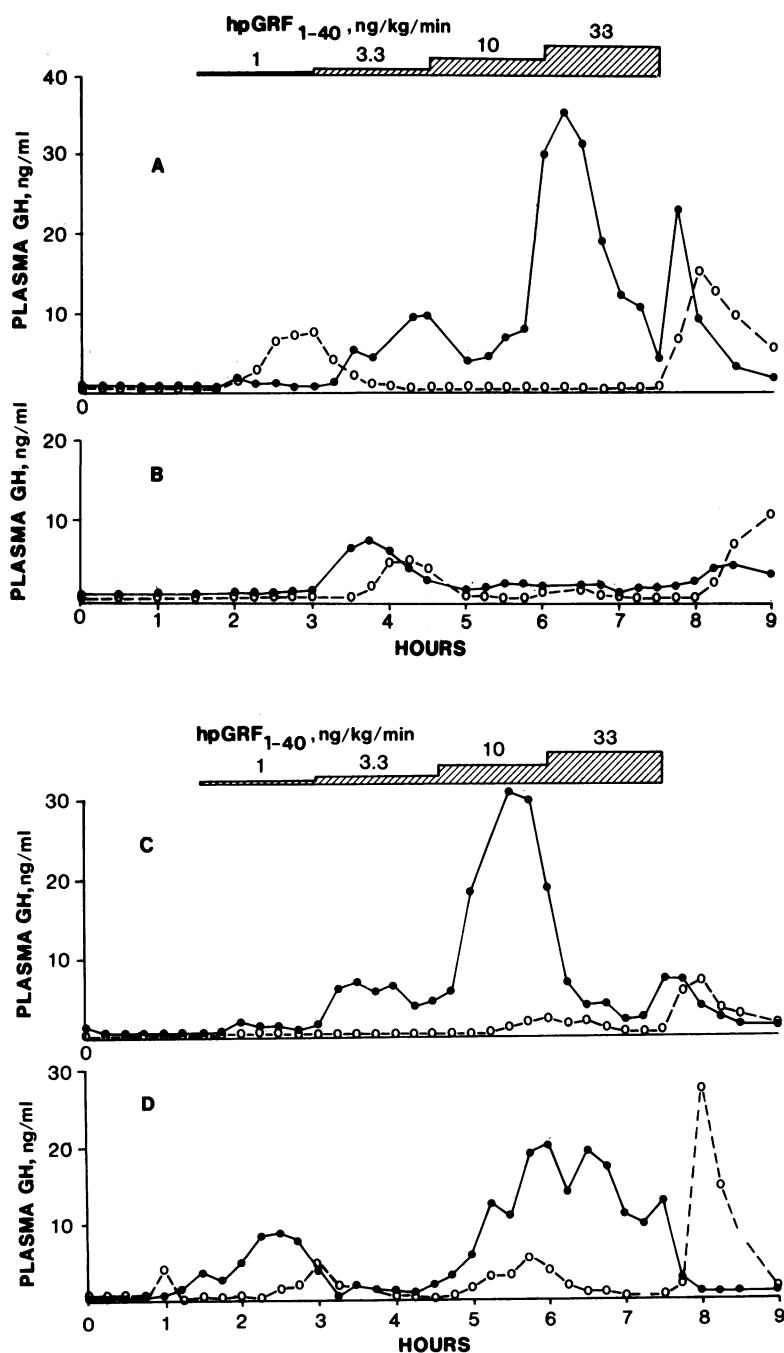


Figure 1. Effect of stepwise increasing infusions of hpGRF(1-40) on plasma GH levels in four normal subjects. The responses to hpGRF(1-40) infusion are shown in the closed circles and continuous lines while those to a saline-control infusion are shown in the open circles and interrupted lines. In both studies, saline was infused during the first and last 90 min. Top: subjects A and B; Bottom: subjects C and D.

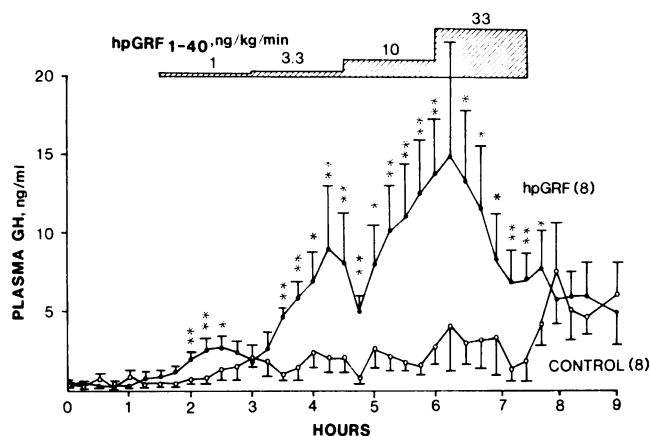


Figure 2. Plasma GH responses to stepwise increasing hpGRF(1-40) infusion. Shown are the mean $\pm$ SE. The asterisks indicate the statistical significance of the difference between hpGRF(1-40) and control infusions as determined by paired *t* test after logarithmic transformation of the GH values. \*, *P* < 0.05; \*\*, *P* < 0.01.

subject D was increased by the 1- and 10-ng/kg per min dose of hpGRF(1-40), though not by the 3.3-ng/kg per min dose. During the 33-ng/kg per min infusion, GH levels were maintained but not further elevated. Of interest is the absence of a post-GRF infusion GH secretory pulse at the time when a spontaneous pulse occurred during the saline control study.

A more consistent pattern of the GH responses to hpGRF(1-40) was apparent when the mean values of the control and experimental studies were compared, as shown in Fig. 2. Despite the individual variability, there was a progressive increase in

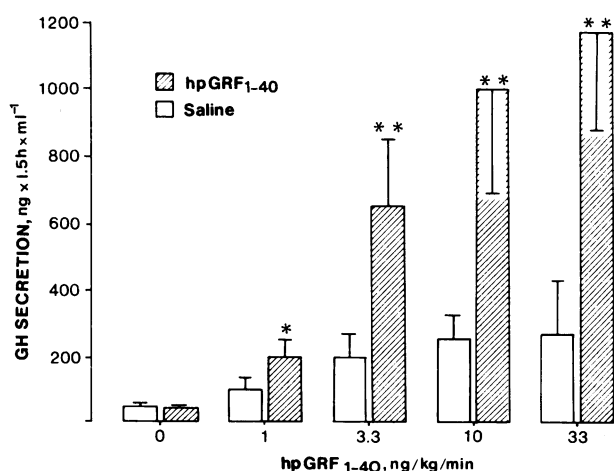


Figure 3. Integrated GH response to hpGRF(1-40) infusions. Shown are the mean $\pm$ SE of the area circumscribed by plasma GH values during sequential 90-min periods in individual subjects receiving hpGRF(1-40) or saline-control infusions. Statistical significance of the difference was determined as in Fig. 2. \*, *P* < 0.02; \*\*, *P* < 0.01.

Table I. Bioactivity of hpGRF(1-40) Infusate in Rat Pituitary Monolayer Cell Culture

Subject	GRF infusate	Relative biopotency*
	ng/kg/min	mean $\pm$ 95% confidence limits
1	1	0.95 (0.43–2.10)
	3.3	0.61 (0.35–1.02)
	33	0.94 (0.72–1.25)
2	1	0.74 (0.40–1.32)
	3.3	1.36 (0.82–2.33)
	33	1.22 (0.48–2.88)

\* Biopotency of infusate was compared to freshly diluted synthetic hpGRF (1-40) standard in terms of rat GH released into the culture media during a 4-h incubation. Infusate and standard were tested at three separate doses by using quadruplicate cultures and potency estimates determined by covariance analysis. A biopotency of 1.00 indicates the infusate bioactivity to be identical to that of the freshly diluted standard.

GH levels during the 1-, 3.3-, and 10-ng/kg per min doses and a gradual decline during the 33-ng/kg per min infusion to levels that were still, however, elevated when compared with those during the control infusion. In all but the 10-ng/kg per min infusion, a decrease in GH levels from peak values occurred at the end of each 90-min infusion. Although the individual GH secretory pulses were no longer discernible, an overall secretory pulse was still evident at 8 h during the control but not the hpGRF(1-40) study.

A quantitative estimation of the GH secretory response to increasing infusion rates of GRF is shown in Fig. 3 where the areas circumscribed by the GH secretion curve during each 90-min period in individual subjects is compared. Highly significant differences were present for each of the infusion rates and the increases were dose responsive from 1 to 10 ng/kg per min. The mean ratios of GRF/saline-control areas for the four hpGRF(1-40) doses from low to high were 1.95, 3.29, 4.29, and 3.65, respectively. Thus, an infusion rate of 1 ng/kg per min was sufficient to double basal GH secretion. During the saline control infusion, GH secretion also increased progressively over

Table II. Plasma IR-GRF Levels during hpGRF(1-40) Infusion

hpGRF(1-40) infusion rate	Plasma IR-GRF at end of 90-min-infusion
ng/kg/min	ng/ml
1	0.35 $\pm$ 0.04* (4)
3.3	1.03 $\pm$ 0.07 (4)
10	3.25 $\pm$ 0.45 (4)
33	8.43 $\pm$ 1.89 (4)

\* Mean $\pm$ SE. Number of subjects is in parentheses.

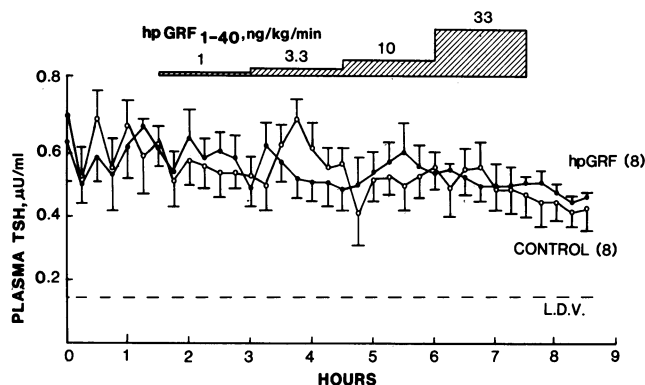


Figure 4. Plasma TSH levels during hpGRF(1-40) and saline-control infusions. Shown are the mean $\pm$ SE. L.D.V., least detectable value.

the period of study, underscoring the need for control studies in individual subjects.

**Measurement of infusate GRF bioactivity and plasma IR-GRF levels.** The bioactivity of hpGRF(1-40) in the infusate from two subjects, assayed in the rat pituitary cell culture system, is indicated in Table I. Each of the infusates was tested at three separate doses; and the results were compared with synthetic standard. The potency of each of the infusates was indistinguishable from that of the standard, which indicated no loss of bioactivity during the infusion.

Plasma IR-GRF levels (mean $\pm$ SE) at the end of each infusion period are shown in Table II. The levels in circulation increased in proportion to the infusion rate throughout the entire dose range used.

**Plasma TSH responses to graded GRF infusion.** Plasma

TSH levels during the hpGRF(1-40) infusion were indistinguishable from those during the saline control infusion, as shown in Fig. 4. In both studies there was a tendency for plasma TSH levels to decrease during the period of observation. The effect of a GRF infusion on the plasma TSH response to TRH is shown in Fig. 5. The infusion of GRF for 1 h before and 1 h after the injection of TRH had no inhibitory effect on the peak TSH response to TRH nor the magnitude of the TSH secretory response. The plasma GH level (mean $\pm$ SE) at the time of TRH injection in the hpGRF(1-40) infusion group was  $10.0\pm2.6$  ng/ml as compared with  $1.0\pm0.3$  ng/ml in the saline infusion group.

**Clinical responses to GRF.** Blood pressure and pulse were monitored throughout the infusions and no significant changes were detected. Three subjects complained of mild headaches at various times beginning 5 h after the start of the graded dose hpGRF(1-40) infusion. One subject also noted a headache during the control study. One subject became nauseated and vomited near the end of the highest dose hpGRF(1-40) infusion and again during the subsequent saline infusion. The characteristic clinical effects of TRH (warmth, micturition urge, metallic taste, and nausea) were unaltered when TRH was given during the hpGRF(1-40) infusion.

## Discussion

The present results clearly indicate that constant infusions of hpGRF(1-40) stimulate GH secretion in normal male subjects and that the response is dose dependent. The maximal GH response was observed at an infusion rate of 10 ng/kg per min, the half-maximal effective dose ( $ED_{50}$ ) was calculated to be 1.9 ng/kg per min and the minimal stimulatory dose was 1 ng/kg per min. Although lower doses were not tested, it is unlikely

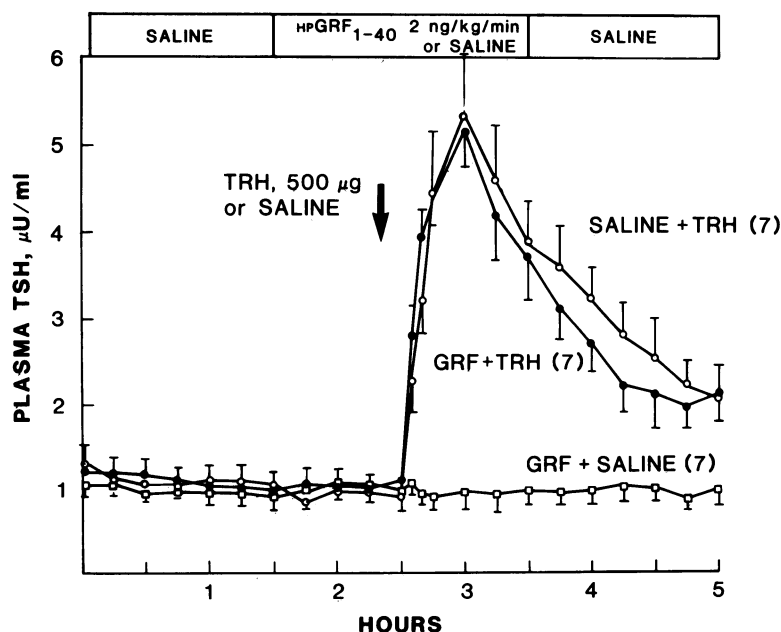


Figure 5. Effect of hpGRF(1-40) infusion on plasma TSH responses to TRH. Shown are the mean $\pm$ SE. All subjects were infused with saline for the initial and final 90-min periods. hpGRF(1-40) ( $\square$  and  $\bullet$ ) or saline ( $\circ$ ) was infused during the 90–210-min period and TRH ( $\bullet$  and  $\circ$ ) or saline ( $\square$ ) injected at 150 min, as indicated.

that significant stimulation could have been detected, given the variability inherent in the responses. Much of the variability could be attributed to spontaneous GH secretory pulses, which raise the possibility of time entrainment of GH secretion within individual subjects. Moreover, integrated GH secretion during the saline-control study did not remain constant, but increased with time to a value six times that during the initial 90-min basal period, at which time no spontaneous pulses were observed. Thus, it was essential to use the saline-control infusion in each subject when analyzing the results.

Inspection of the mean GH responses and even more strikingly, the individual responses, offered unequivocal evidence of intermittent secretion or of attenuation of the GH response. This was seen in individual subjects irrespective of dose and in the entire group, most prominently at the highest dose studied. Although the design of the present study did not permit a differentiation between the effects of time and dose, several possible explanations for the findings merit consideration.

First, it could be argued that pituitary GH content was significantly depleted by the time of the highest dose hpGRF(1-40) infusion. Constant infusions of hpGRF(1-40) in rats result in a similar qualitative GH secretory pattern, which has been attributed to depletion of pituitary GH stores (16). The quantity of GH secreted in response to the lower three doses can be determined by multiplying the integrated secretion area, shown in Fig. 3, ( $\text{ng} \cdot \text{min} \cdot \text{ml}^{-1}$ ) by the GH metabolic clearance rate ( $190 \text{ ml} \times \text{min}^{-1}$ ) (17). GH secretion, calculated in this manner ( $\sim 370 \mu\text{g}$ ), represents only a small fraction of the total pituitary GH content (10–15 mg) (18) and therefore cannot be considered to represent significant depletion.

Second, the possibility exists that a selective intracellular pool of GH within the somatotroph, susceptible to GRF-stimulated release rather than total somatotroph GH, was depleted. Multiple pools of GH have been shown to exist in the somatotroph (19), which can be differentially stimulated by various secretagogues (20). An argument against this hypothesis, however, is derived from the observation that GRF is capable of stimulating the release of nearly 50% of the GH contained in rat pituitary monolayer cultures during a 4-h incubation period (21). Unless marked differences exist between rat and human somatotrophs and between *in vitro* and *in vivo* models, selective pool depletion is unlikely as an explanation for the attenuation.

A third possibility considered for the lack of response to the top infusion dose was that of degradation of hpGRF(1-40) during infusion or enhanced metabolic clearance, which resulted in reduced bioactive GRF levels in circulation. This was excluded by the demonstration that (a) GRF bioactivity of the infusate was completely preserved at the end of the infusion, and (b) plasma IR-GRF levels rose in proportion to the infusate concentration.

A fourth and physiologically more plausible explanation is that the elevated plasma GH levels stimulated hypothalamic somatostatin release which, in turn, inhibited the GH response to GRF. Refractoriness of GH secretion to repeated stimulation by L-dopa (22) or sequential exercise and arginine infusion (23)

has been previously demonstrated. Furthermore, exogenous GH administration inhibits spontaneous pulsatile endogenous GH secretion in the rat (24) and inhibits nyctohemeral (25) and insulin- (26), arginine-, or exercise-induced (23) GH secretion in man by unknown but possibly somatostatin-mediated mechanisms. GH stimulates somatostatin release from the rat hypothalamus *in vitro* within 20 min at a concentration of 20 ng/ml (8). We made no attempt to measure plasma somatostatin levels, however, since peripheral circulating somatostatin is believed to originate from sites other than the hypothalamus.

Evidence for a somatostatin-mediated inhibition of the GH response to GRF was initially sought by measuring plasma TSH levels during the graded GRF infusion on the assumption that an increase in hypothalamic somatostatin release might suppress basal TSH secretion. Although the TSH assay exhibited sufficient sensitivity to detect reductions of up to 75% in basal TSH levels and is capable of distinguishing between basal TSH levels in normal and thyrotoxic subjects, no differences were observed between hpGRF(1-40) and saline-control studies. Since, in the initial studies, intermittent secretion was observed at doses as low as 1 ng/kg per min, we infused hpGRF(1-40) at a rate of 2 ng/kg per min, which should have been sufficient to stimulate endogenous hypothalamic somatostatin release. TRH-stimulated TSH secretion was chosen as a possibly more sensitive system to demonstrate inhibition by somatostatin. Once again, there was no inhibition of the TSH response and thus no evidence to implicate increased somatostatin secretion as the mechanism responsible for the attenuation of the GH response. It must be acknowledged that the sensitivity of the somatotroph as compared with the thyrotroph to inhibition by somatostatin has not yet been systematically determined and it is conceivable that somatotroph inhibition could occur at a hypothalamic-hypophyseal portal plasma concentration to which the thyrotroph is insensitive.

As a fifth alternative, it is possible that impaired GH responsiveness to continuous hpGRF(1-40) infusion involves a cellular mechanism at the level of the somatotroph. Whether this represents receptor down-regulation or a postreceptor phenomenon is unknown, though there is precedence to support the former alternative. Quantitative reduction of pituitary receptors to gonadotropin-releasing hormone (GnRH) (27), corticotropin releasing factor (28), dopamine (29), and somatostatin (30) have been demonstrated after exogenous administration or physiologic perturbations, which result in enhanced endogenous secretion. It is therefore not unreasonable that a similar mechanism exists with respect to the GRF receptor. The data presented make a strong argument in favor of a homeostatic regulatory phenomenon. However, the syndrome of acromegaly associated with ectopic GRF secretion suggests that the phenomenon is modulatory rather than absolute, in contrast to studies with GnRH where gonadotropin secretion can be abolished by continuous GnRH administration.

Finally, consideration must be given to the occurrence of spontaneous GH secretory pulses during the period of hpGRF(1-40) infusion, above and beyond the difficulty encountered in

distinguishing them from responses to exogenous GRF. This is most clearly seen in Fig. 1, subject A, where a spontaneous pulse occurred immediately before the start of the highest infusion rate. The most likely basis for their occurrence is a response to endogenous GRF secretion that very likely occurs in a pulsatile manner as does the secretion of other releasing hormones (31). If so, the concentration of hypothalamic-hypophyseal portal GRF must be quite high at these times, i.e., at least 5–10 ng/ml, to elicit a GH response greater than that due to the exogenous infusion. Alternatively, the endogenous stimulus could consist of a different GRF with much greater affinity than that of hpGRF(1–40) toward the somatotroph GRF receptor or acting through a separate receptor. Identification of the precise structure of GRF in the human hypothalamus and of the GRF released into the portal system will be required to resolve this issue. Another perplexing issue raised by the present data relates to the persistence of endogenous GRF secretion in the presence of exogenous GRF infusion and elevated plasma GH levels. This question cannot be answered at the present time. The possibility that the GH pulses are attributable to rebound secretion subsequent to a decrease in hypothalamic somatostatin release is less likely on the basis of the previous discussion and the fact that spontaneous GH pulses (in the rat) occur even when somatostatin tone is removed by anti-somatostatin serum treatment (32). The implications of these findings as related to the feedback regulation of hypothalamic GRF secretion remain to be explored.

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