

Suppression of growth hormone (GH) secretion by a selective GHRH-releasing hormone (GHRH) antagonist. Direct evidence for involvement of endogenous GHRH in the generation of GH pulses.

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Research Article

To study the potential involvement of growth hormone-releasing hormone (GHRH) in the generation of growth hormone (GH) pulses in humans we have used a competitive antagonist to the GHRH receptor, (N-Ac-Tyr¹,D-Arg²)GHRH(1-29)NH₂(GHRH-Ant). Six healthy young men were given a bolus injection of GHRH-Ant 400 micrograms/kg body wt or vehicle at 2200 h and nocturnal GH concentrations were assessed by every 10-min blood sampling until 0800 h. Integrated total and pulsatile GH secretion were suppressed during GHRH-Ant treatment by 40 ± 6 (SE) % and 75 ± 5%, respectively. GHRH-Ant suppressed maximum (7.6 ± 2.2 vs 1.8 ± 0.5 micrograms/liter; P < 0.001) and mean (3.3 ± 1.0 vs 1.1 ± 0.2 micrograms/liter; P = 0.02) GH pulse amplitudes. There was no change in integrated nonpulsatile GH levels, pulse frequency, or interpulse GH concentration. GHRH-Ant 400 micrograms/kg also suppressed the GH responses to intravenous boluses of GHRH 0.33 micrograms/kg given 1, 6, 12, and 24 h later by 95, 81, 59, and 4%, respectively. In five healthy men, the responses to 10-fold larger GHRH boluses (3.3 micrograms/kg) were suppressed by 82 and 0%, 1 and 6 h after GHRH-Ant 400 micrograms/kg, respectively. These studies provide the first direct evidence that endogenous GHRH participates in the generation of spontaneous GH pulses in humans.

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Suppression of Growth Hormone (GH) Secretion by a Selective GH-releasing Hormone (GHRH) Antagonist

Direct Evidence for Involvement of Endogenous GHRH in the Generation of GH Pulses

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Abstract

To study the potential involvement of growth hormone-releasing hormone (GHRH) in the generation of growth hormone (GH) pulses in humans we have used a competitive antagonist to the GHRH receptor, (*N*-Ac-Tyr¹,*D*-Arg²)GHRH(1-29)NH₂(GHRH-Ant). Six healthy young men were given a bolus injection of GHRH-Ant 400 µg/kg body wt or vehicle at 2200 h and nocturnal GH concentrations were assessed by every 10-min blood sampling until 0800 h. Integrated total and pulsatile GH secretion were suppressed during GHRH-Ant treatment by 40±6 (SE) % and 75±5%, respectively. GHRH-Ant suppressed maximum (7.6±2.2 vs 1.8±0.5 µg/liter; *P* < 0.001) and mean (3.3±1.0 vs 1.1±0.2 µg/liter; *P* = 0.02) GH pulse amplitudes. There was no change in integrated non-pulsatile GH levels, pulse frequency, or interpulse GH concentration. GHRH-Ant 400 µg/kg also suppressed the GH responses to intravenous boluses of GHRH 0.33 µg/kg given 1, 6, 12, and 24 h later by 95, 81, 59, and 4%, respectively. In five healthy men, the responses to 10-fold larger GHRH boluses (3.3 µg/kg) were suppressed by 82 and 0%, 1 and 6 h after GHRH-Ant 400 µg/kg, respectively. These studies provide the first direct evidence that endogenous GHRH participates in the generation of spontaneous GH pulses in humans. (*J. Clin. Invest.* 1993. 92:695-701.) Key words: hypothalamus • pituitary • human • pulsatility • neuroendocrine

Introduction

Growth hormone (GH)¹ secretion is pulsatile in all species studied thus far. GH release appears to be under dual control by two hypothalamic peptides, GH-releasing hormone (GHRH) and somatostatin (SRIF), which promote and inhibit GH secretion, respectively. The importance of GHRH pulses as generators of pulsatile GH secretion has been directly studied in vivo in several animal species. In the rat, both immunoneutralization of GHRH (1, 2) and abolition of GHRH secretion (3, 4) eliminate GH pulses, whereas administration of SRIF antibody increases interpulse GH concentrations (1,

5). Direct sampling of rat (1) or sheep (6) hypophysial-portal blood has found GHRH peaks to be largely concurrent with GH pulses, validating the analysis of GH pulsatility as a method for the study of endogenous GHRH secretion in these species.

Multiple human studies have assessed GH pulsatility in various physiologic conditions, such as puberty (7), aging (8), fasting (9), and the menstrual cycle (10), as well as in human illnesses such as growth delay (11), acromegaly (12, 13), diabetes (14), and obesity (15). The underlying assumption in the interpretation of these experiments has been that in humans, as in rats and sheep, GH pulses reflect periodic discharges of hypothalamic GHRH, whereas interpulse GH levels are set by tonic SRIF secretion. However, whether GHRH plays such a role in the generation of pulsatile GH secretion in humans is unknown. Peripheral blood concentrations of GHRH (16) or SRIF (17) do not reflect pituitary portal blood levels and the direct approaches that have been used in animal studies are not practical in humans. Since there are large species-specific differences in the neuroendocrine control of GH secretion, animal data cannot be directly extrapolated to humans. For example, acute hypoglycemia results in a decrease in GH secretion in rats (18) and sheep (6), whereas the same stimulus increases GH secretion in humans (19). Calorie deprivation increases GH secretion in sheep (20, 21) and humans (9) but decreases it in the rat (22). Importantly, normal humans (23) and even GHRH-deficient children (24, 25) given continuous GHRH infusions exhibit highly pulsatile GH secretion. This implies that another stimulus, such as periodic SRIF withdrawal, may govern the generation of GH pulses and indeed, acute withdrawal of SRIF infusion does elicit acute GH response in humans (26, 27). Establishment of the role of GHRH in the genesis of GH pulses in humans is crucial for the understanding of both normal GH secretory physiology and the neuroendocrine mechanism operative in various disease states.

(*N*-Ac-Tyr¹,*D*-Arg²)GHRH(1-29)NH₂(GHRH-Ant) is an analogue of GHRH that functions as a competitive antagonist at the level of the GHRH receptor. This was demonstrated both in rat pituitary cells (28) and in COS cells transfected with the cDNA for the human GHRH receptor (29). It does not alter baseline GH secretion (30) but abolishes GHRH effects upon adenylate cyclase activity (28, 29) and GH release (29-32). Administration of GHRH-Ant to rats eliminates both GH pulses and somatic growth (30). We have used this compound to determine whether endogenous GHRH is involved in the generation of spontaneous GH pulses in humans.

Methods

Subjects and study design. The study was approved by the Food and Drug Administration, the University of Michigan Institutional Review Board, and the Clinical Research Center Review Committee. All sub-

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1. Abbreviations used in this paper: GH, growth hormone; GHRH, GH-releasing hormone; GHRH-Ant, (*N*-Ac-Tyr¹, *D*-Arg²)GHRH(1-29)NH₂; SRIF, somatostatin.

jects involved in this study signed an informed consent document. All were nonsmoking males, 20–34 yr of age, who were taking no medications and had unremarkable medical history and physical examinations. All had normal height (1.72–1.88 m) and body mass index (20–26 kg/m²). Screening work-ups, which included serum electrolytes, glucose, calcium, phosphorus, biochemical indices of renal and hepatic function, and a complete blood count, were normal in all subjects.

In every experiment, each subject was studied twice in the Clinical Research Center in an open labeled fashion, once during administration of the vehicle, normal saline, and once while receiving GHRH-Ant. There was a minimum of 4 d between the completion of saline studies and the beginning of GHRH-Ant treatment days.

In experiment 1, subjects were admitted into the Clinical Research Center at 1600 h, at which time an intravenous (iv) catheter was placed in a forearm vein of one arm for blood sampling and a second catheter was placed in a forearm vein of the other arm for drug administrations. Meals were served at 0700, 1300, and 1800 h and there was no between meal snacking. Water was allowed ad lib. Throughout the study, subjects were allowed to walk freely in the CRC, but refrained from vigorous activity. Lights were turned on at 0700 h and off at 2300 h. Day-time napping was not permitted.

Since most spontaneous GH secretory episodes occur at night, the effect of GHRH-Ant on spontaneous pulsatile GH secretion was assessed during the nighttime hours. In contrast, the ability of GHRH-Ant to block the GH secretory effects of exogenous GHRH was tested during the waking hours as spontaneous GH pulses, which might interfere with data interpretation, would be less likely to occur. At 2200 h either 20 ml of saline or GHRH-Ant 100 µg/kg of body weight was given intravenously over 3 min and blood pressure, heart rate, and temperature were closely monitored for the next hour. Plasma cortisol, luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and prolactin were measured before and 30, 60, and 90 min after the first dose of GHRH-Ant. Blood sampling for GH was performed every 10 min from 2000 h on the day of admission until 0800 h the following morning, at which time the same dose of saline or GHRH-Ant was given. GHRH 0.33 µg/kg iv bolus was given 1, 6, 12, and 24 h after the second dose of saline or GHRH-Ant. Blood samples were obtained every 20 min, from 1 h before until 2 h after each GHRH dose. Blood was also collected for repeat measurements of the screening blood tests at baseline and then 1 and 24 h after the first dose of GHRH-Ant.

This experiment was done in two individuals only. As will be presented below, nocturnal pulsatile plasma GH concentrations in these subjects were only partially suppressed with GHRH-Ant 100 µg/kg. For this reason, subsequent experiments were performed using GHRH-Ant at the dose 400 µg/kg of body weight. In experiment 2, GHRH-Ant was administered at this higher dose to six subjects, otherwise the protocol was identical to that performed in experiment 1.

In experiment 3, five subjects were admitted into the CRC at 0730 h at which time an iv catheter was placed into a forearm vein. At 0900 h they were given saline or GHRH-Ant 400 µg/kg iv over 6–8 min. Intravenous boluses of GHRH 3.3 µg/kg were given 1 and 6 h later (1000 and 1500 h). Blood samples were obtained every 10 min from 0830 until 0900 h and every 20 min between 0900 and 1200 h and between 1400 and 1700 h, encompassing 1 h before and 2 h after each GHRH injection. Subjects were given breakfast at 0730 h and lunch at 1200. The same limitations on between meal snacking and activity as in the previous experiments were applied.

Materials. GHRH(1-44) was purchased from Bachem Inc. (Torrance, CA) and was prepared by the University of Michigan Investigational Pharmacy to a concentration of 50 µg/ml. GHRH-Ant was purchased from Bachem Bioscience (Philadelphia, PA) and was prepared to a concentration of 1 or 5 mg/ml in 0.9% saline.

Assays. Plasma GH was measured in duplicate by an established radioimmunoassay (33) with materials generously donated by the National Institute of Diabetes and Digestive and Kidney Diseases Pituitary and Hormone Distribution Program. In experiments 1 and 2, all samples from each subject were run in the same assay. All samples from

experiment 3 were run simultaneously. Mean intrassay coefficient of variation of replicate samples was below 8% in each assay and mean assay sensitivity was 0.27 µg/liter. GH concentrations below assay sensitivity were assigned the value of assay sensitivity before data analysis. All other assays were performed by the Hematology, Chemistry, and Ligand Laboratories (Department of Pathology, University of Michigan Medical Center) using established methodologies.

Data analysis. Parameters of pulsatile GH concentration during the 2200 h–0800 h period were analyzed by the computer program Cluster (34) using a power fit, a *t* statistic of 2 and a cluster size of 2 × 2 (35). To minimize detection of false positive pulses, only computer identified pulses that were greater in amplitude (nadir to peak) than assay sensitivity were considered as true pulses. Interpulse GH was the mean GH concentration between pulses. Only segments that were both identified by Cluster as nonpulsatile GH secretion and were more than 1 half-life of GH (20 min) away from the end of the preceding pulse were included in the estimation of interpulse GH concentration. Integrated total GH concentration (µg × min/liter) was calculated as the area under the GH versus time curve (AUC) using the trapezoidal rule. Integrated pulsatile GH concentration (µg × min/liter) was defined as the area under the GH versus time curve during time segments identified as a pulse by Cluster minus the contribution of the baseline. Baseline was calculated by the trapezoidal rule, using GH values at the beginning and the end of a pulse. Integrated nonpulsatile GH (µg × min/liter) was measured as integrated total minus the integrated pulsatile GH concentrations. Similarly, the GH responses to bolus GHRH were defined both in terms of the absolute increase from nadir to peak (µg/liter) as well as the pulsatile area under the GH versus time curve after GHRH administration.

ANOVA for repeat measures and two-tailed paired or unpaired Student's *t* tests were used when appropriate for statistical comparisons between groups. If the data were not normally distributed, they were logarithmically transformed before analysis. All results are presented as mean ± standard error (M ± SE) and *P* < 0.05 was considered significant.

Results

During the baseline studies, nocturnal GH profiles were pulsatile in all eight subjects who were studied in experiments 1 and 2. Between 1 and 4 GH pulses were detected in each individual (2.8 ± 0.4 pulses/10 h), with a mean pulse amplitude of 7.7 ± 3.5 µg/liter. Administration of exogenous GHRH (0.33 µg/kg) produced a GH response of similar magnitude (6.9 ± 1.4 µg/liter; *P* = 0.76). In the first two subjects, who received GHRH-Ant 100 µg/kg, plasma GH response to GHRH 0.33 µg/kg was suppressed by a mean of 91, 62, 53, and 0%, 1, 6, 12, and 24 h later (data not shown). Their nocturnal GH profiles during saline and GHRH-Ant treatment are shown in Fig. 1. The integrated total and pulsatile GH concentrations were suppressed by an average of 47 and 53%, respectively.

In experiment 2, we used a larger dose of GHRH-Ant, 400 µg/kg, in an attempt to more completely block both the effect of exogenous GHRH and spontaneous nocturnal GH pulsatility. The effects of GHRH-Ant 400 µg/kg on GH responses to GHRH are presented in Fig. 2. Nearly complete (95 ± 2%; GHRH vs saline; *P* = 0.0001) suppression was achieved when GHRH was administered 1 h after GHRH-Ant and there was a gradual disappearance of the suppressive effect of GHRH-Ant on GH responses thereafter: 81 ± 3% at 6 h (*P* = 0.0001), 59 ± 12% at 12 h (*P* = 0.0014), and 4 ± 30% at 24 h (*P* = 0.49).

The nocturnal GH profiles of the six volunteers who received GHRH-Ant 400 µg/kg or saline at 2200 h are presented in Fig. 3. The mean (±SE) GH values of the same six subjects

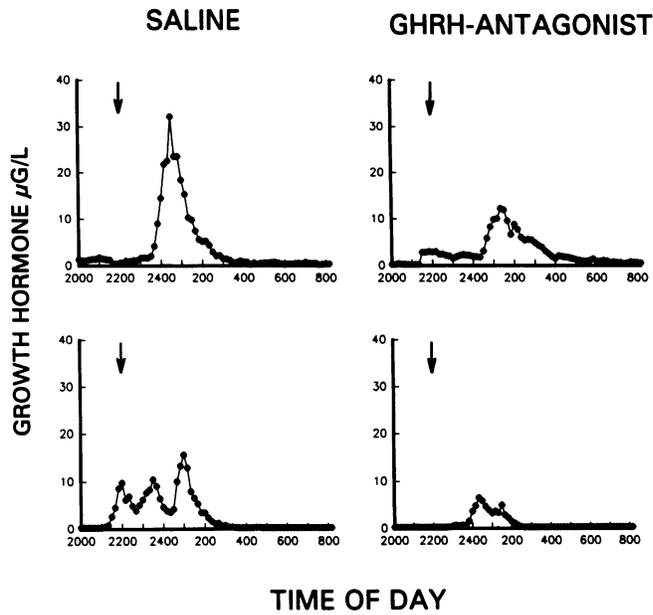


Figure 1. Individual GH concentration profiles of the two subjects who received GHRH-Ant 100 $\mu\text{g}/\text{kg}$. Saline or GHRH-Ant was administered at 2200 h and blood sampling was performed every 10 min.

during the overnight sampling are shown in Fig. 2. Integrated total and pulsatile GH concentrations were suppressed by GHRH-Ant during the 10-h time period by 40 ± 6 and $75\pm 5\%$, respectively. The effects of GHRH-Ant on discrete parameters of GH secretion in these six subjects are given in Table I. There was no change in pulse frequency, however both mean and maximum pulse amplitude were markedly decreased during GHRH-Ant treatment. There were strong correlations between the magnitude of spontaneous GH secretion during saline secretion and the residual GH secretion during GHRH-Ant for

both integrated total ($r = 0.88$; $P = 0.017$) and pulsatile ($r = 0.92$; $P = 0.001$) GH concentrations. Since there was no change in the nonpulsatile component of the GH profile, the suppression of the integrated total GH concentration during GHRH-Ant treatment could be attributed entirely to its effect upon the pulsatile component of the profile.

In experiment 3, the ability of GHRH-Ant 400 $\mu\text{g}/\text{kg}$ to block the effect of a 10-fold higher dose of GHRH was tested. During control studies, intravenous boluses of GHRH 3.3 $\mu\text{g}/\text{kg}$ produced increases in plasma GH of 8.3 ± 2.4 and 18.1 ± 5.3 $\mu\text{g}/\text{liter}$ 1 and 6 h after a saline bolus, respectively. Although the response at 1 h was similar to that obtained with GHRH 0.33 $\mu\text{g}/\text{kg}$ 1 h after saline bolus in experiment 1 (7.1 ± 3.6 $\mu\text{g}/\text{liter}$; $P = 0.65$), the response to the larger dose of GHRH at 6 h was significantly greater than that obtained with GHRH 0.33 $\mu\text{g}/\text{kg}$ (5.3 ± 2.8 $\mu\text{g}/\text{liter}$; $P = 0.01$). As shown in Fig. 4, plasma GH response to GHRH 3.3 $\mu\text{g}/\text{kg}$ at 1 h was antagonized to a large degree by GHRH-Ant, but not as completely as the response to GHRH 0.33 $\mu\text{g}/\text{kg}$ ($81\pm 3\%$ vs $95\pm 2\%$; GHRH 3.3 $\mu\text{g}/\text{kg}$ vs GHRH 0.33 $\mu\text{g}/\text{kg}$; $P = 0.0017$). However, this dose of GHRH-Ant was completely ineffective in inhibiting GH response to GHRH 3.3 $\mu\text{g}/\text{kg}$ given 6 h later ($1,795\pm 532$ $\mu\text{g} \times \text{min}/\text{liter}$ vs $1,439\pm 400$ $\mu\text{g} \times \text{min}/\text{liter}$; GHRH-Ant vs sal; $P = 0.57$).

GHRH-Ant was well tolerated in all subjects. After acute administration of the drug, there were no significant changes in blood pressure, heart rate, or body temperature. All of the subjects who received the dose of GHRH-Ant 400 $\mu\text{g}/\text{kg}$ experienced a hot flush that began within 5 min of drug administration and lasted from 5 to 40 min. Most, but not all subjects developed erythema that was most prominent on the face, neck, torso, and arms. The erythema lasted from 10 to 40 min and was generally less intense after the second dose of GHRH-Ant. Neither of the two subjects who received GHRH-Ant 100 $\mu\text{g}/\text{kg}$ had either hot flush or erythema. None of the subjects developed shortness of breath, wheezing, conjunctival or soft

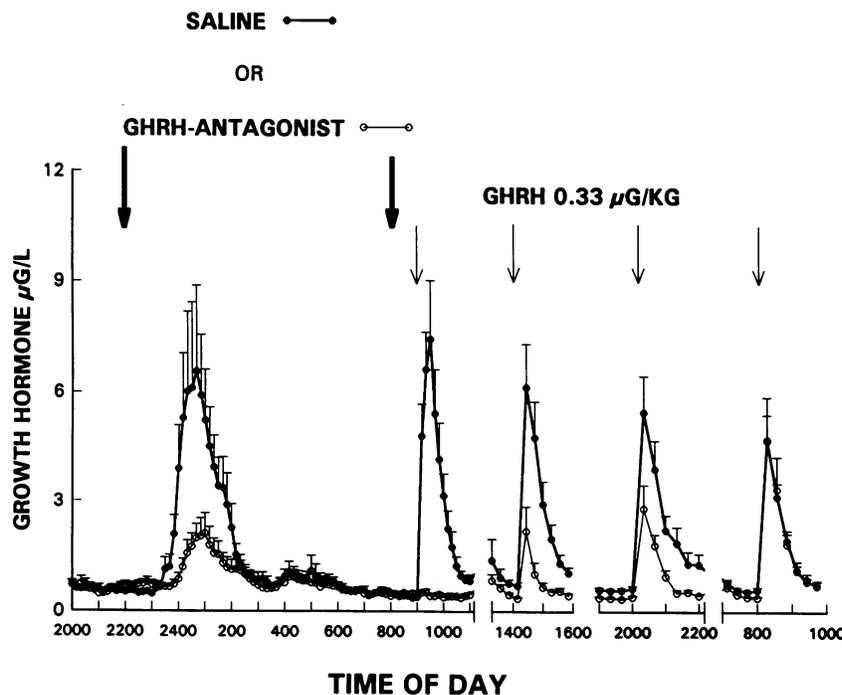


Figure 2. Effects of GHRH-Ant 400 $\mu\text{g}/\text{kg}$ on pulsatile GH concentration and GH response to GHRH 0.33 $\mu\text{g}/\text{kg}$. Saline or GHRH-Ant (heavy arrows) was given at 2200 h and 0800 h. GHRH boluses (light arrows) were given 1, 6, 12, and 24 h after the second bolus of saline or GHRH-Ant. Data are shown as the mean \pm SE; $n = 6$.

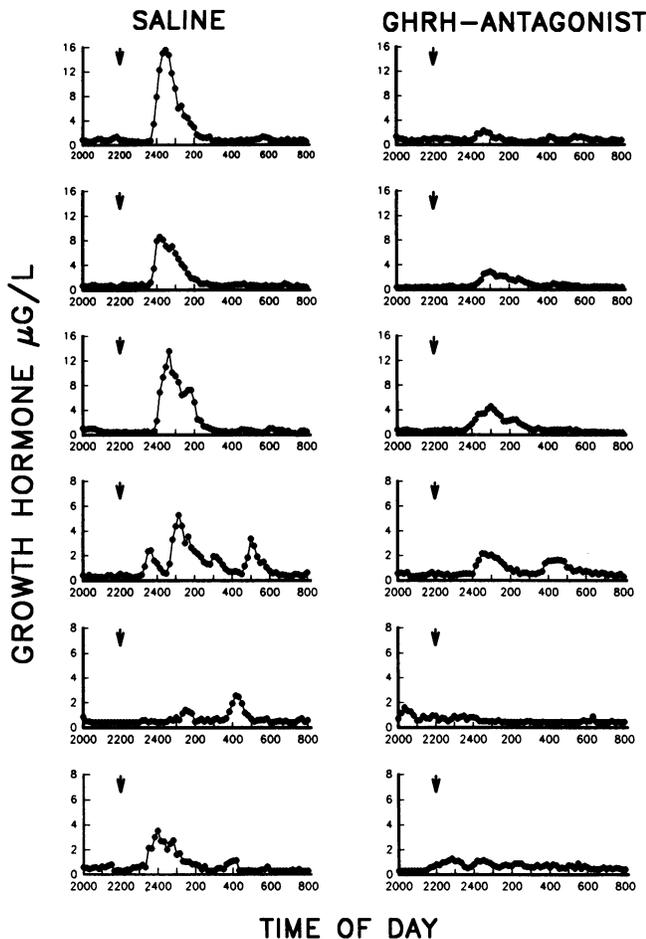


Figure 3. Individual nocturnal GH concentration profiles of the six subjects who received GHRH-Ant 400 $\mu\text{g}/\text{kg}$. GHRH-Ant was administered at 2200 h and blood sampling was performed every 10 min.

tissue swelling, pruritic urticaria, or generalized pruritis. Five subjects were scratch skin tested between 2 and 8 wk after receiving GHRH-Ant. All had positive control responses to codeine and none had a reaction to GHRH-Ant. There were no changes in the hematologic or biochemical blood tests and none developed eosinophilia. There were no acute changes in plasma cortisol, TSH, LH, FSH, or prolactin concentrations after GHRH-Ant administration.

Discussion

Present understanding of the neuroendocrine control of GH secretion has primarily come from direct studies in nonhuman species. Chemical (4) or surgical (3) ablation of the GHRH neurons in the arcuate nucleus or immunoneutralization of GHRH (1, 2) all result in attenuation or cessation of pulsatile GH secretion. The presence of high GHRH concentrations in the pituitary portal blood of rats (1) and sheep (6) at the time of GH pulses directly indicates the central role of GHRH in GH pulse generation in these species. However, in the sheep, only 62% of GH pulses coincided with GHRH secretory episodes (6), suggesting that a non-GHRH-mediated mechanism may also be operative.

The neuroendocrine origins of GH pulsatility in humans are not known since the paradigms used in animal studies

Table 1. Effect of GHRH-Ant 400 $\mu\text{g}/\text{kg}$ on Discrete Parameters of Pulsatile GH Concentration

Parameter	Saline	GHRH-Ant	P
Integrated total GH ($\mu\text{g} \times \text{min}/\text{liter}$ per 10 h)	965 \pm 182	529 \pm 64	0.005
Integrated pulsatile GH ($\mu\text{g} \times \text{min}/\text{liter}$ per 10 h)	633 \pm 172	172 \pm 55	0.019
Integrated nonpulsatile GH ($\mu\text{g} \times \text{min}/\text{liter}$ per 10 h)	332 \pm 25	357 \pm 17	0.43
Interpulse GH ($\mu\text{g}/\text{liter}$)	0.50 \pm 0.05	0.59 \pm 0.05	0.24
Pulse frequency (pulses per 10 h)	3.1 \pm 0.4	2.3 \pm 0.4	0.14
Maximum pulse amplitude ($\mu\text{g}/\text{liter}$)	7.6 \pm 2.2	1.8 \pm 0.5	<0.001
Mean pulse amplitude ($\mu\text{g}/\text{liter}$)	3.3 \pm 1.0	1.1 \pm 0.2	0.02

Every 10-min blood sampling was performed between 2200 and 0800 h in six healthy men. Normal saline or GHRH-Ant 400 $\mu\text{g}/\text{kg}$ was administered at 2200 h. Data are shown as the mean \pm standard error (M \pm SE) and are analyzed by paired Student's *t* test.

are impractical in human investigation. The introduction of a selective GHRH receptor antagonist, (*N*-Ac-Tyr¹,*D*-Arg²)GHRH(1-29)NH₂, provided a novel approach to study physiology of GH secretion in humans. Our data demonstrate that this compound decreases the magnitude of both GHRH-induced as well as spontaneous GH pulses in humans. This is the first direct evidence of GHRH participation in the generation of spontaneous GH pulsatility in humans.

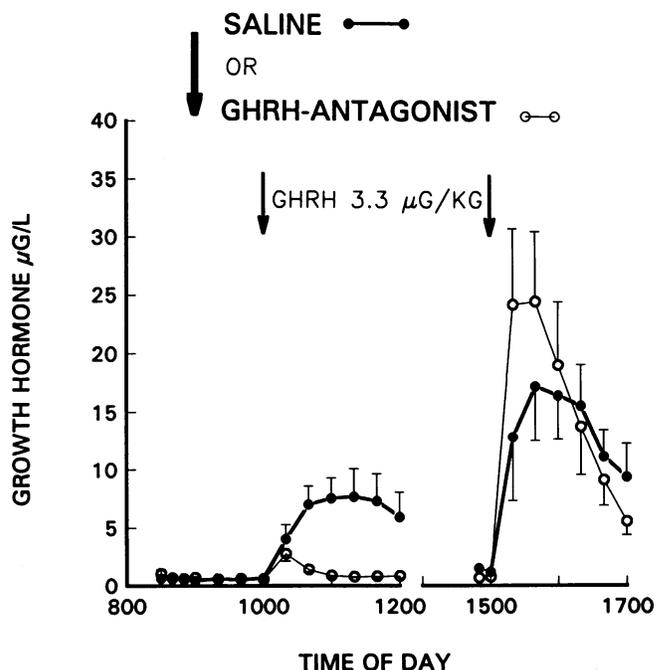


Figure 4. Effect of GHRH-Ant 400 $\mu\text{g}/\text{kg}$ on the GH response to GHRH 3.3 $\mu\text{g}/\text{kg}$. Saline or GHRH-Ant (heavy arrow) was given at 0900 h and GHRH boluses (light arrows) were given 1 and 6 h later. Data are shown as mean \pm SE; *n* = 5.

To test the capacity of GHRH-Ant to counteract GHRH action *in vivo*, we assessed the inhibitory effect of this compound upon GH release induced by exogenous GHRH. We used a GHRH dose of 0.33 $\mu\text{g}/\text{kg}$ since in previous studies (36–38) similar doses resulted in a half-maximal response and produced GH pulses of the same magnitude as spontaneously occurring pulses in humans (12, 38). This dose in humans results in plasma GHRH concentrations similar to the GHRH concentrations needed for half-maximal GH stimulation *in vitro* (36, 39) and agrees with data on GHRH receptor binding affinity (39). In our experiments, we found that GH responses to GHRH 0.33 $\mu\text{g}/\text{kg}$ were similar in magnitude to the spontaneous GH pulses, suggesting that this dose, as has been concluded by others (38), was physiological. Although there was no difference between the GH responses to GHRH 0.33 $\mu\text{g}/\text{kg}$ and GHRH 3.3 $\mu\text{g}/\text{kg}$ 1 h after saline injection, the response at 6 h was considerably greater after the larger dose of GHRH. The difference between responses at the two times during control treatment was likely due to both high inter- and intrasubject variability of GH responses to GHRH (36, 37) and modest group sizes. A single *iv* bolus of GHRH-Ant 400 $\mu\text{g}/\text{kg}$ almost totally blocked the stimulatory effect of GHRH 0.33 $\mu\text{g}/\text{kg}$ at 1 h and this effect gradually diminished with time. The same dose of GHRH-Ant, however, was less effective against a 10-fold larger dose of GHRH and its effect dissipated rather quickly. These data support previous *in vitro* and *in vivo* animal studies (28–32) validating this compound as a competitive GHRH receptor antagonist.

Despite nearly complete suppression of the GH response to GHRH 0.33 $\mu\text{g}/\text{kg}$, GHRH-Ant only partially inhibited nocturnal pulsatile GH concentrations. First, it is possible that the effect of a single bolus of GHRH-Ant had decreased by the time of peak nocturnal GH secretion. Whereas almost complete suppression of GH response to GHRH 0.33 $\mu\text{g}/\text{kg}$ occurred 1 h after GHRH-Ant, this effect declined to 81% after 6 h. Peak nocturnal GH concentration occurred on the average of 2.5 h after the administration of GHRH-Ant. Therefore, some decrement in activity would have been expected during the time period in which pulsatile GH concentrations were measured. Second, it is possible that hypophysial-portal blood GHRH levels during nocturnal pulses are higher than those achieved after an *iv* bolus of GHRH 0.33 $\mu\text{g}/\text{kg}$ and overcome the inhibiting effects of GHRH-Ant. Although the dose of GHRH-Ant 400 $\mu\text{g}/\text{kg}$ fully suppressed the response to GHRH 0.33 $\mu\text{g}/\text{kg}$, it was insufficient to prevent the GH response to a 10-fold higher dose of GHRH. In addition, the nearly 100% suppression of the effect of GHRH 0.33 $\mu\text{g}/\text{kg}$ at 1 h might partially reflect residual activity from the GHRH-Ant dose that was given 12 h earlier. Furthermore, we observed a strong correlation between nocturnal integrated GH concentrations during saline and GHRH-Ant treatments. One possible explanation for this finding may be the relationship between the magnitude of endogenous GHRH secretion and the resultant GH secretion, whereby the fixed dose of GHRH-Ant might not suppress GH secretion as completely in subjects with high endogenous GHRH (presumably reflected by high GH pulses) as in those with low endogenous GHRH secretion. These observations suggest that GHRH-Ant, either in a dose greater than 400 $\mu\text{g}/\text{kg}$ or as a continuous infusion, might be needed to overcome nocturnal secretion of GHRH. Third, there is the possibility of a non-GHRH component in nocturnal GH release. One or more of the recently described peptides,

such as pituitary adenylate cyclase activating polypeptide (40), galanin (41, 42), or the putative endogenous ligand for the family of growth hormone-releasing peptides (43) could be secreted concurrently with GHRH. Additionally, nocturnal decline in hypothalamic SRIF secretion could either itself promote GH secretion or result in increased somatotroph sensitivity to GHRH.

The importance of SRIF in the generation of pulsatile GH secretion is unknown. The persistence of pulsatile GH secretion in both normal men (23) and children with GHRH deficiency (24, 25) during continuous GHRH infusions, which presumably negate the possible contribution of endogenous GHRH, suggests that intermittent declines in endogenous SRIF secretion might play a role in the generation of GH pulses. This is supported by the demonstration of a fall in hypophysial-portal SRIF before and during periods of GH secretion in the rat (1) and by acute GH release after SRIF withdrawal *in vitro* (44–46), and *in vivo* in both rats (47, 48) and humans (26, 27). This effect could be expressed directly at the pituitary level, either by itself or in conjunction with a concomitant GHRH pulse. Since GHRH is responsible for GH synthesis (49) it is conceivable that periodic declines in SRIF secretion might produce GH pulses simply by allowing the stored pituitary GH to be released. In this scenario, suppression of GH secretion by GHRH-Ant would be due to limited GH synthesis, not an inhibition of GH release. This, however, is unlikely since pituitary GH content far exceeds the amount of GH released in our short-term experiment (9, 50).

Additionally, GH secretion associated with SRIF withdrawal may involve the acute release of GHRH. Anatomical data demonstrating direct connections between SRIF-containing neurons and GHRH-immunoreactive cells of the arcuate nucleus (51–54) support the central regulation of GHRH by SRIF. SRIF might tonically inhibit hypothalamic GHRH release (1, 55, 56) and GHRH antibody prevents the rebound GH secretion associated with both SRIF immunoneutralization (56) and withdrawal of a SRIF infusion (48). Indeed, the rebound GH secretion associated with SRIF withdrawal is less prominent *in vitro* than *in vivo* (45, 57) and in humans, the rebound pulses occur less consistently and are smaller than after GHRH boluses (26, 27). Moreover, Frohman et al. found no correlation between hypophysial-portal SRIF secretion and either GH or GHRH release in the sheep (6). Although our data do not exclude either periodic, nocturnal declines in SRIF secretion or the participation of non-GHRH hypothalamic factors as contributing mechanisms, the fact that a single dose of GHRH-Ant suppressed pulsatile GH concentrations by 75% indicates that endogenous GHRH plays the major role in determining nocturnal GH release in humans.

The role of GHRH in determining interpulse GH concentrations is uncertain. GHRH immunoneutralization in male rats has no effect on interpulse GH concentration (1, 56); however, elimination of GHRH action (58, 59) or secretion (60) decreases interpulse GH in females. Thus, SRIF may not be the only determinant of tonic GH secretion. We have found no changes in GH interpulse levels during antagonist administration, cautiously suggesting that GHRH may not be important in the determination of interpulse GH levels in young men. This conclusion must be tempered by the realization that many of the interpulse GH levels were at or below assay sensitivity. Similarly, the same technical limitation has precluded us from addressing the effects of GHRH-Ant on GH “micropulses”

(61), that might have been hidden below our RIA detection limit. Thus, at the present time it remains uncertain whether GHRH-Ant selectively inhibits all GH pulses or reduces overall GH secretion. These questions will be better resolved with the introduction of more sensitive GH assays and by the assessment of discrete parameters of GH pulsatility using continuous GHRH-Ant infusions during the daytime when large GH pulses do not usually occur. Additionally, sex related differences of the effect of GHRH-Ant will have to be ascertained.

In conclusion, we have provided the first direct evidence for GHRH involvement in the regulation of GH secretion, including the generation of spontaneous GH pulses in humans. Furthermore, our study validates the use of GHRH antagonist as a novel paradigm to study mechanisms of GH secretion in humans. This approach will allow more precise understanding of the neuroendocrine mechanisms involved in the regulation of GH secretion in normal and pathologic states.

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