Induction of growth hormone (GH) mRNA by pulsatile GH-releasing hormone in rats is pattern specific

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Borski, Russell J., Wellington Tsai, Roberta DeMott-Friberg, and Ariel L. Barkan. Induction of growth hormone (GH) mRNA by pulsatile GH-releasing hormone in rats is pattern specific. Am J Physiol Endocrinol Metab 278: E885–E891, 2000.—Growth hormone-releasing hormone (GHRH) is a main inducer of growth hormone (GH) pulses in most species studied to date. There is no information regarding the pattern of GHRH secretion as a regulator of GH gene expression. We investigated the roles of the parameters of exogenous GHRH administration (frequency, amplitude, and total amount) upon induction of pituitary GH mRNA, GH content, and somatic growth in the female rat. Continuous GHRH infusions were ineffective in altering GH mRNA levels, GH stores, or weight gain. Changing GHRH pulse amplitude between 4, 8, and 16 µg/kg at a constant frequency (Q3.0 h) was only moderately effective in augmenting GH mRNA levels, whereas the 8 µg/kg and 16 µg/kg dosages stimulated weight gain by as much as 60%. When given at a 1.5-h frequency, GHRH doubled the amount of GH mRNA, elevated pituitary GH stores, and stimulated body weight gain. In the rat model, pulsatile but not continuous GHRH administration is effective in inducing pituitary GH mRNA and GH content as well as somatic growth. These studies suggest that the greater growth rate, pituitary mRNA levels, and GH stores seen in male compared with female rats are likely mediated, in part, by the endogenous episodic GHRH secretory pattern present in males.

SOMATIC GROWTH DEPENDS ON a multitude of complex and often interrelated mechanisms, including genetic factors, nutrition, and adequate hormone milieu. Among these factors, growth hormone (GH) secretion by somatotrophs is central to regulating growth. In all vertebrates studied to date, GH is secreted in a pulsatile fashion (for review see Ref. 13). The episodic pattern of GH release is thought to be central in regulating a variety of GH-mediated processes, including hepatic epidermal growth factor receptors, corticosteroid binding globulins, and serum cholesterol, apoprotein, and lipoprotein concentrations (18, 22, 24, 32). In the adult rat, the markedly higher growth rates of males compared with females is regulated, in part, by the more episodic pattern of GH secretion seen in males (7, 19, 30).

Growth hormone secretion in male rats is characterized by secretory bursts that occur every 3–3.3 h with low, almost undetectable levels between peaks, whereas females have a more continuous secretion with substantially higher baseline GH levels (11, 38). The pulsatile pattern of GH secretion is governed mainly by GH-releasing hormone (GHRH) and somatostatin (SRIF). Studies in rats with use of antiserum to either peptide or GHRH antagonist demonstrate that GHRH induces GH pulses, whereas low interpulse GH levels are maintained by SRIF (29, 34, 37). Indeed, both GHRH and SRIF are thought to be secreted 180 degrees out of phase, leading to the highly episodic pattern of GH secretion in males (37). In the intact adult female rat, administration of exogenous GHRH every 3 h imitates the male pattern of GH secretion and promoted growth, whereas continuous GHRH administration in the same dose was less effective (7). This study clearly demonstrates that the manner of GHRH stimulation may be more important than the total amount in promoting somatic growth.

In addition to greater growth rates, adult male rats have higher pituitary GH mRNA levels, pituitary GH content, and elevated circulating insulin-like growth factor I (IGF-I) levels compared with age-matched females (17, 40). Although the pattern of GHRH administration is a major determinant of episodic GH secretion and somatic growth, there is little information with regard to its in vivo regulation of GH gene expression. The present investigation was undertaken to determine whether GHRH pulse amplitude and/or frequency might be critical to masculinizing several aspects of the somatotrophic axis of intact female rat. To this end, we evaluated whether the pattern of GHRH when administered in a pulsatile male-like fashion might be a primary mediator of the elevated pituitary GH mRNA levels, pituitary GH stores, plasma IGF-I concentrations, and greater growth rates one sees in male compared with female rats.

MATERIALS AND METHODS

Animals and experimental procedures. The protocol was approved by the Animal Studies Committee of the Veterans Affairs Medical Center and the University of Michigan Medical Center, Ann Arbor, Michigan 48109.
Affairs Medical Center. Male (12 wk old) and young female Sprague-Dawley rats (6 wk old, 140–170 g) were purchased from Charles River Laboratories (Wilmington, MA) and housed separately in individual cages and acclimated for 3–4 days in a temperature (22°C), humidity-, and light-controlled (12:12-h light-dark cycle, lights on between 0600 and 1800) environment before surgery. Animals were given unlimited access to water and food (Purina Rat Chow, Richmond, IN) before and throughout the experiment. Animals were anesthetized under ketamine-xylazine (87 and 13 mg/kg sc, respectively), and a right-jugular venous catheter was implanted and exteriorized through a plastic cap and spring (to protect catheter) attached between the shoulder blades. The spring was connected to a swivel, permitting continuous venous infusion or sampling and allowing free movement within the cage. The catheter consisted of PE 50 tubing tipped on the venous end with 1.5-cm Silastic medical grade tubing (0.012 in. ID, 0.025 in. OD). Animals were weighed, returned to their cages, and allowed to recover before infusions. Only those animals that attained presurgical body weight or resumed growth were used in the experiments. At the start of the experiment, animals were weighed and then infused (Autosyringe AS2C) with human GHRH-(1–44) intravenously (Bachem, Torrance, CA) continuously or in a series of pulses every 3, 6, and 9 h at total daily doses of 32, 64, or 128 µg/kg (Bachem, Torrance, CA) continuously or in a series of pulses every 3, 6, and 9 h at total daily doses of 32, 64, or 128 µg/kg (Bachem, Torrance, CA) continuously or in a series of pulses (17, 33). The blots were prehybridized in 5× SSPE, 5× Denhardt’s solution, 0.5% SDS, and 100 µg/ml of sonicated denatured herring sperm DNA) for 2 h at 50°C and then incubated overnight in fresh buffer containing newly labeled GH cDNA probe at 50°C. Filters were washed two times in 2× SSPE at room temperature for 10 min and then one time with 1× SSPE at 50°C for 15 min. The number of counts per spot was determined with a phosphomager (Ambis, Vernon Hills, IL). Specific binding was determined by subtracting nonspecific counts (a spot to which no RNA and only denaturing buffer was added) from total counts per spot. The concentration of GH mRNA was calculated as picograms of GH cDNA probe bound per 100 micrograms of pituitary DNA, according to an equation previously described (17). Pools of pituitary cytosolic total RNA (100 ng/spot) were applied in triplicate to each filter to control for differences in hybridization between filters [coefficient of variation (CV) <10%]. CV between individual replicates was <8%. No significant signal above background was measured in 10-µg aliquots of TRNA or liver total RNA confirming the specificity of the GH cDNA for pituitary GH mRNA that had been previously demonstrated by Northern analysis in our laboratory (17).

Plasma hormone determinations. Plasma and pituitary cytosolic GH levels were measured separately by RIA against the reference standard preparation rGH-RP-2 by using materials obtained from the NIDDK, as previously described (23). All samples were run in duplicate in a single assay. The coefficients of variation between replicates were <10% for both plasma and pituitary cytosolic GH RIAS (assay sensitivity = 1.85 ng/ml). Plasma IGF-I samples were measured in triplicate in a single assay by RIA after acid-ethanol extraction (10) with antiserum (provided by Drs. J. J. Van Wyk and L. Underwood and distributed by the National Hormone and Pituitary Program, NIDDK) and recombinant human IGF-I (Mallinkrodt, St. Louis, MO) as a standard. Intra-assay CV was <10% (assay sensitivity = 108 ng/ml).

Statistical analysis. Differences among means were evaluated by use of one-way ANOVA followed by Fisher’s least
RESULTS

Male and female GH secretory profiles. Before evaluating the dosages of GHRH that induce male-like GH pulses in female rats, we examined the typical GH blood profiles of each sex. Figure 1 shows the typical daily GH secretory pattern of young female rats that we used in our subsequent studies and of adult male rats. Animals were sampled every 30 min. The male GH profile is characterized by high amplitude pulses that reach ∼180 ng/ml on average. In some individual animals, pulses were >600 ng/ml. These pulses occur every 3 h and are interspersed by low basal GH levels. Compared with males, females show a relatively apul- satile GH secretory pattern with low amplitude, irregu- lar peaks, and overall higher basal GH concentrations.

Dose-response effect of GHRH on GH secretion. We verified that the GHRH concentrations used in female rats were those that produce physiological GH pulses typical of the endogenous male GH secretory pattern. Therefore, effects of the first injection of GHRH were measured in a subset of individuals from the GHRH pulsatility experiment. Female rats were injected intravenously with 4, 8, or 16 µg/kg of GHRH, and GH was measured from blood collected 5 min later. There was a clear dose-response relationship between the amount of GHRH injected and plasma GH levels (Table 1). The lowest 4 µg/kg dose increased plasma GH concentration by ∼260 ng/ml, which is typical of the rise seen in males during an endogenous pulse (Fig. 1). Administration of the highest GHRH dose (16 µg/kg) resulted in GH concentrations of 620 ng/ml, which falls in the range of endogenous pulse peaks measured in individual adult males. It was difficult to maintain catheter patency for blood withdrawal without persistently disturbing animals or interrupting the varied patterns of GHRH administration; therefore, we did not routinely measure GH secretory responses to GHRH over the course of the experiment. However, collection of trunk blood 5 min after the last injection of the 8 µg/kg of GHRH dosage revealed plasma GH levels virtually identical to those after the first injection. This suggests that the female rat continues to show one-on-one GH secretory bursts with no refractoriness in response to bolus GHRH injections, even after 7 days. This confirms an earlier finding showing that GH secretory bursts occur in response to each GHRH bolus injection with no desensitization for up to 12 consecutive days of treat- ment (7).

Pattern of GHRH administration on pituitary GH mRNA and GH stores. To determine whether the pattern of GHRH administration is an important determin- ant of pituitary GH mRNA expression, 6-wk-old female rats were given GHRH as a continuous infusion or as bolus injections of 4–16 µg/kg every 3 h or 8 µg/kg of GHRH every 1.5, 3, or 6 h. Continuous infusion of GHRH at daily doses ranging from 32 to 128 µg/kg did not significantly alter GH mRNA levels compared with animals infused with saline (Fig. 2). Administration every 3 h of 8 µg/kg of GHRH significantly elevated GH mRNA levels above those seen in pituitaries of animals receiving continuous infusion of saline or GHRH at the same total daily dosage of 64 µg/kg (P < 0.05). Changing the frequency of GHRH administration also significa- ntly altered GH mRNA levels. Injection of 8 µg/kg of GHRH every 90 min caused a twofold increase in GH mRNA levels compared with animals infused with saline (Fig. 2). Administration every 3 h of 8 µg/kg of GHRH significantly elevated GH mRNA levels above those seen in pituitaries of animals receiving continuous infusion of saline or GHRH at the same total daily dosage of 64 µg/kg (P < 0.05). Changing the frequency of GHRH administration also significantly altered GH mRNA levels. Injection of 8 µg/kg of GHRH every 90 min caused a twofold increase in GH mRNA levels compared with animals receiving saline control infusions (P < 0.001), continuous GHRH infusion (P < 0.001), or a bolus GHRH injection of 16 µg/kg every 3 h at the same daily dose of 128 µg/kg (P < 0.01). Like GH mRNA, pituitary GH stores were also altered by the pattern of GHRH administration (Fig. 2). Bolus injection of 8 µg/kg of GHRH every 90 min increased pituitary GH content over levels measured in animals receiving saline (P < 0.05), continuous GHRH infusion (P < 0.01), or bolus GHRH injections every 3 h (P = 0.07) at the same total daily dose of 128 µg/kg. Injection of 4–16 µg/kg at a constant frequency of 3 h or continuous infusion of 32–128 µg/kg of GHRH did not alter pituitary GH content from that seen in saline infused animals.

Table 1. Effect of varying doses of GHRH on plasma GH levels 5 min after intravenous injection

<table>
<thead>
<tr>
<th>GHRH Dosage</th>
<th>First Injection</th>
<th>Last Injection</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>5 min</td>
</tr>
<tr>
<td>Saline</td>
<td>69.9 ± 11.3</td>
<td>36.2 ± 6.8</td>
</tr>
<tr>
<td>4 µg/kg bw</td>
<td>44.1 ± 6.42</td>
<td>300.7 ± 65.6</td>
</tr>
<tr>
<td>8 µg/kg bw</td>
<td>69.7 ± 15.8</td>
<td>469.0 ± 47</td>
</tr>
<tr>
<td>16 µg/kg bw</td>
<td>10.7 ± 4.41</td>
<td>620.0 ± 156</td>
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</tbody>
</table>

Data are from the first and last bolus intravenous injection given to animals treated over a 7-day period with bolus growth hormone-releasing hormone (GHRH) injections. Values are means ± SE; n = 5–19. Data following the last GHRH injections are shown only for those animals receiving saline and 8 µg/kg bolus GHRH injections.

significance difference test for predetermined comparisons (36). Data are shown as means ± SE.

Fig. 1. Typical daily growth hormone (GH) secretory profiles from 6-wk-old immature female and 12-wk-old adult male rats sampled over a 9-h period. Each point represents average GH concentration from 6 different animals.
Pattern of GHRH administration on somatic growth and serum IGF-I concentrations. Continuous infusion of GHRH at total daily doses of 32, 64, and 128 µg/kg did not alter weight gain compared with saline infused animals (Fig. 3). Delivery of increasing doses of 8 and 16 µg/kg of GHRH at a constant frequency of 3 h increased somatic growth compared with those animals receiving either a continuous infusion of saline or GHRH at equivalent total daily GHRH dosages. Likewise, delivery of 8 µg/kg of GHRH at different frequencies (every 1.5, 3, or 6 h) significantly enhanced somatic growth compared with those animals receiving a continuous infusion of saline or GHRH at the same total daily dose.

To evaluate whether changes observed in somatic growth might be mediated by alterations in hepatic IGF-I function, circulating IGF-I levels were also measured in animals receiving different patterns of GHRH administration. The changes observed in somatic growth were not accompanied by alterations in circulating IGF-I (Fig. 3). There were no differences in plasma IGF-I concentrations between animals receiving saline or GHRH, regardless of the mode of GHRH delivery.

DISCUSSION

An earlier study clearly showed that administration of human GHRH to immature female rats at 40-, 90-, or 180-min frequencies produced one-on-one, male-like GH secretory bursts with no desensitization in the GH response (8). Due to its uniformity in GH responses to exogenous GHRH and relatively hypopulsatile spontaneous GH secretory profile (11, 21, 37), the female rat provides an appropriate model for assessing the role that endogenous GHRH pulsatility plays in regulating...
the somatotrophic axis. This is the first report demonstrating that pituitary steady-state GH mRNA levels are dependent, in part, on the pattern of GHRH secretion. We found that pulsatile administration of GHRH is more effective than a continuous mode of delivery in inducing pituitary GH mRNA accumulation and GH protein stores as well as promoting somatic growth in female rats. The doses of GHRH used in this study produced acute GH responses within the amplitude range of endogenous GH pulses measured in adult male rats (see Fig. 1 and Table 1). Thus the degree of GHRH stimulation was likely within the physiological range.

Compared with females, male rats possess higher pituitary GH and GH mRNA levels and exhibit plasma GH secretory profiles with high-amplitude peaks that occur at 3-h frequencies interspersed with low, almost undetectable, interpeak GH levels (11, 15, 17, 21, 40). The latter is likely due to the highly pulsatile pattern of hypothalamic GHRH secretion, because GH pulses are abolished in animals and humans treated with GHRH antisemur or GHRH receptor antagonist (20, 29, 34, 37). The importance of the pulse pattern of neurohormone secretion on gene induction of its respective pituitary target hormone(s) has been clearly demonstrated for gonadotropin-releasing hormone and follicle-stimulating hormone/luteinizing hormone (14). Whether the same is true for GHRH-GH interactions is unknown. The present study suggests that, in addition to effects on GH secretion, pulsatile GHRH is central for inducing elevations in GH mRNA and GH content and could be responsible, in part, for the higher levels observed in pituitaries of male compared with female rats. We found that bolus injections of GHRH every 1.5 h, or at a 3.0-h frequency to mimic the male pattern of endogenous GHRH secretion, increased pituitary GH mRNA levels over those animals receiving similar total daily dosages of GHRH as a continuous infusion (Fig. 2). Moreover, rapid (Q1.5h) GHRH pulse delivery significantly elevated pituitary GH stores in parallel with its induction of GH mRNA. Interestingly, “physiological” (Q3.0h) GHRH pulses increased GH mRNA but not pituitary GH content, suggesting a different time course effect of GHRH on these parameters. Indeed, a similar dosage of GHRH-(1—29)NH₂ (=9 µg/kg body wt of GHRH-(1—44), delivered at 3-h frequencies for 12 days, elevated pituitary GH stores in female rats of a similar age and size to those used in this study (7). Exposure to dihydrotestosterone (DHT) increases pituitary GH stores and mRNA levels in ovariectomized female rats in vivo, whereas the opposite occurred when animals were treated with estrogen (6). Because DHT, but not estrogen, increased hypothalamic GHRH mRNA and peptide expression (1, 15), this serves as additional support for the crucial role played by pulsatile GHRH in augmentation of GH synthesis and sexually dimorphic expression of pituitary mRNA and protein stores. Our studies, however, do not rule out a potentially important role for pulsatile SRIF in modulating GHRH-induced sex differences in GH mRNA expression. Nevertheless, it appears GHRH may induce GH synthesis by acting directly on the somatotroph, because it was previously shown to stimulate GH gene transcription, GH mRNA accumulation, and GH stores in pituitary cell cultures (3, 4, 12).

The frequency rather than the amplitude component of GHRH pulses is more effective in elevating pituitary GH mRNA and GH content, whereas either is sufficient for inducing somatic growth. We found that injections of GHRH at 1.5-h intervals increased pituitary GH mRNA and GH content over levels observed in animals given the same daily dosage at 3-h intervals, whereas both frequencies similarly stimulated weight gain. It is possible that GHRH may augment pituitary GH and GH mRNA stores via increased GH synthesis in a manner distinct from its stimulatory effect on GH secretion and somatic growth. GHRH was shown to stimulate GH gene transcription independently of its induction of GH release in primary pituitary cell cultures (3). The physiological relevance of enhanced GH mRNA and GH stores seen with GHRH pulse frequencies beyond the 3-h intervals typically observed in male rats is unclear; nevertheless, our findings suggest that GHRH delivery at more rapid pulse frequencies could serve the additive effect of enhancing growth while simultaneously stimulating GH synthesis in GH-deficient subjects. Future studies should address whether GHRH pulse frequency might be a mediator of in vivo somatotroph hyperplasia and proliferation, which accompany the pathophysiological conditions associated with GHRH hypersecretion (2, 5, 25, 31).

Pulsatile GHRH treatment increased body weight gain by as much as 60%, whereas the same total daily dose administered as a continuous infusion for 7 days had no effect (Fig. 3), confirming an earlier study (7). This potent action (8% body wt gain/day) of GHRH is independent of the frequency or amplitude of GHRH administration as long as the peptide is given in a pulsatile fashion. Previous studies have shown that deprivation of GHRH or treatment with GHRH antagonist, conditions that abolish plasma GH secretory peaks, impair body weight gain (7, 17, 25, 29). Likewise, intermittent delivery of GHRH to hypophysectomized rats was shown to be more effective than a continuous GH infusion in stimulating growth (19, 30). Consistent with these observations, our results demonstrate that GH secretory spikes, derived mainly through episodic increases in GHRH secretion, are central to mediating the greater growth rates observed in male compared with female rats.

Despite its stimulation of body weight gain, pulsatile GHRH did not significantly alter circulating IGF-I in this study (Fig. 3). These findings differ from that of a previous report (26), where chronic administration of GHRH antagonist for 2 wk caused a small albeit significant decline in circulating IGF-I in female rats. Because hepatic IGF-I production is dependent on GH, it is not surprising that the inhibition of endogenous GH secretion with GHRH antagonist would reduce circulating IGF-I. However, our results suggest that additional increases in plasma GH after exogenous GHRH treatment may not suffice to increase hepatic, and therefore plasma IGF-I, above levels already ele-
vated by endogenous GH present in normal animals. Recent studies using molecular approaches to selectively knock out IGF-I gene expression in the liver indicate that the paracrine, rather than endocrine, source of IGF-I is critical for postnatal body growth (35, 39, 41). We postulate that pulsatile GHRH delivery, by generating an episodic pattern of GH secretion, enhances somatic growth by preferentially inducing IGF-I production at peripheral tissues rather than at the liver. This hypothesis is supported by a previous investigation showing that pulsatile GH treatment is more effective than continuous GH infusion in stimulating IGF-I mRNA levels in rib growth plate and skeletal muscles, two major targets for the growth-promoting actions of GH (19).

In summary, our results show for the first time that pulsatile GHRH administration is more effective than continuous infusion in elevating pituitary GH mRNA levels. Increases in pituitary GH mRNA levels were accompanied by similar rises in GH content, suggesting that pulsatile GHRH may be an important inducer of GH gene expression and GH synthesis in vivo. Although all parameters of pulsatile GHRH delivery stimulate growth, the frequency rather than the amplitude component was more effective in inducing pituitary GH stores and gene expression. Exogenous GHRH did not alter circulating IGF-I concentrations, regardless of the pattern of delivery. The lack of concordance between somatic growth and serum IGF-I levels after continuous infusion of an amidated fragment of human growth hormone suggests that growth hormone mRNA and GH secretion in male and female rats: regulation by gonadal steroids. Primary effect on insulin-like growth factor I gene expression and secretion. Endocrinology 137: 3253–3259, 1996.


