Growth-hormone-releasing hormone (GHRH, somatoliberin) is the hypothalamic peptide hormone that specifically stimulates synthesis and release of growth hormone (GH, somatotropin) by somatotrope cells of the anterior pituitary gland. GHRH is the last of the classically postulated hypothalamic hormones to be characterized, synthesized, and used in clinical medicine. In this review of GHRH, I discuss the discovery and characterization of the peptide, its role in the regulation of GH secretion, and its clinical use in pathological states of GH excess and GH deficiency. The two most clinically useful aspects of GHRH are to establish the etiology of GH deficiency, most commonly the result of a hypothalamic GHRH deficiency, and to treat GH-deficient children. Use of GHRH as therapy for GH deficiency currently is experimental and, to date, results encourage the idea of a therapeutic role for this peptide in promoting endogenous GH secretion with resulting acceleration of linear growth.

Additional Keyphrases: somatoliberin · somatotropin · growth-hormone deficiency · regulation of growth hormone secretion

Growth-hormone-releasing hormone (GHRH, somatoliberin) is the last of the classical hypothalamic hormones to be identified and characterized. The existence of such substances was postulated by Green and Harris in 1947 (1). The other known hypothalamic regulatory hormones include several peptides—thyrotropin-releasing hormone (thyroliberin), gonadotropin-releasing hormone (GnRH, gonadoliberin), corticotropin-releasing hormone (corticoliberin), and somatostatin (SRIF)—and the monoamine, dopamine. Except for somatostatin and dopamine, these compounds specifically stimulate the release of specific pituitary hormones. The characterization and synthesis of these hypothalamic hormones and of their agonists and antagonists have greatly aided the study of both normal and abnormal neuroendocrine physiology, resulting in the detection and treatment of several hypothalamic–pituitary disorders. This discussion is directed to GHRH, its discovery, biological properties, and clinical usefulness.

Growth hormone (GH, somatotropin) secretion by the anterior pituitary gland is regulated by two hypothalamic hormones: GHRH, which stimulates its release, and somatostatin, which inhibits its release. GHRH is secreted episodically in bursts that can be detected by frequent measurement of GH concentrations in normal subjects and by measurement of GHRH concentrations in patients with ectopic GHRH-producing tumors. When blood is sampled as often as every 20 min or every 5 min during 24 h, one can determine total secretion and characterize secretory profiles. Studies of normal men and women have demonstrated that most GH secretion occurs in bursts at night (2). The burst of GH probably is a result of the concomitant secretion of GHRH and withdrawal of somatostatin release by the hypothalamus (3). The interaction of these hormones and other modulators of GH release is being studied by numerous groups.

Historical Perspective

GHRH was isolated, sequenced, and synthesized in 1982 by two independent groups, one led by Vale (4), the other by Guillemin (5). The method of discovery of GHRH was unique in that all work was carried out with human pancreatic tumors that secreted GHRH and caused acromegaly. In contrast, the other hypothalamic hormones had been characterized by using thousands of hypothalami from animals. The GHRH peptide in the tumor used by the Vale group was found to consist of 40 amino acids (4); the tumor used by the Guillemin group contained three peptides with 37, 40, and 44 amino acid residues (5). The 1–37 and 1–40 amino acids of the GHRH isolated by Vale and colleagues were homologous with the first 37 and 40 amino acids of the 1–44 GHRH isolated by Guillemin and colleagues. Additionally, 64% of the GHRH in the tumor used by the Guillemin group was 1–40, 12% was 1–37, and 22% was 1–44, which makes it unlikely that the 1–40 form is a degradation product of the 1–44 form (6). In vivo studies have demonstrated that the 37-, 40-, and 44-amino-acid peptides are equipotent in stimulating GH release (7). However, the full biological activity of GHRH peptides isolated from both tumors resides in the 29 residues ending at the amino terminus (4, 5). The stimulatory effect of GHRH on GH release occurs via stimulation of cyclic AMP production (8, 9).

Isolation of messenger RNA from both tumors led to the development of cDNA probes (10, 11). Subsequently, Mayo et al. (12) sequenced the human GHRH gene, located on chromosome 20, and determined that it spans 10 kilobases and contains five exons.

The sequence of GHRH from other animals has also been determined. Rat hypothalamic GHRH consists of 43 amino acids and has a free carboxyl terminus (13). This peptide differs from the human 1–44 in 15 amino acid substitutions or deletions. Porcine (14) and bovine (15) hypothalamic GHRH are 44 amino acids long and are amidated at the carboxyl terminus.

Although GHRH was originally isolated from an extra-hypothalamic site, subsequent studies demonstrated its presence in the hypothalamic nuclei of human and primate brains. GHRH-containing neurons have been identified by immunostaining in the infundibular and ventromedial

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1 Nonstandard abbreviations: GH, growth hormone; GHRH, growth-hormone-releasing hormone; and GnRH, gonadotropin-releasing hormone.

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nuclei (16-18). GHRH immunoreactivity also has been detected in the human pancreas, placenta, brain (other than hypothalamus), and gut (19-21). The presence of GHRH in the gut may be of clinical importance because radioimmunoassayable GHRH is present in the serum of patients with ectopic GHRH secretion, in normal subjects, and in GH-deficient patients who are presumably deficient in hypothalamic GHRH (22-25). Thus, detection of a 100- to 1000-fold increase in GHRH concentrations (i.e., microgram per liter concentrations vs the normal nanograms per liter) in a patient with acromegaly is useful to determine whether the patient has an ectopic GHRH-secreting tumor. However, measurable GHRH in normal subjects and in GH-deficient patients probably reflects gastrointestinal secretion rather than hypothalamic secretion. GHRH concentrations in the plasma of normal subjects may range from <10 to 300 ng/L, depending upon the specific radioimmunoassay and antibody; commercial GHRH assays are not yet available. Additionally, GHRH has been detected by immunocytochemical staining in several types of neuroendocrine tumors, including pancreatic, bronchial carcinoid, gut carcinoid, thymic carcinoid, medullary carcinoma of the thyroid, pheochromocytoma, and small-cell carcinoma of the lung (26). However, only two of the 24 patients studied had clinical features of acromegaly, suggesting that the peptide was not biologically active. Similarly, immunoreactive GHRH has been detected in pheochromocytomas, ganglioneuroblastomas, medullary carcinoma of the thyroid, insulinomas, gastrinomas, glucagonomas, small-cell carcinomas, and vasoactive intestinal peptide-secreting tumors (19). Although several types of neuroendocrine tumors may synthesize GHRH, the associated clinical syndrome of acromegaly is rare.

GHRH Effects on GH Secretion

Initial studies with GHRH were designed to determine its ability to stimulate GH release, its specificity, its clinical effects and safety, the likelihood of a dose–response relationship, and its indirect effects on the generation of insulin-like growth factor I (somatomedin C). GHRH, as GHRH-40, was first administered to healthy male volunteers in December 1982 (27). Six young men were given GHRH-40 (1 μg per kilogram body weight) intravenously, and the concentrations of GH and other pituitary hormones were measured and compared with the concentrations after placebo administration. On the control day (placebo), two subjects had small spontaneous GH peaks; after receiving GHRH, all had increased concentrations of GH in their serum. This increase varied among the subjects, which probably reflects variable release of endogenous hypothalamic somatostatin to diminish GH secretion (Figure 1) (27). GHRH was specific in stimulating GH release; there was no effect on other anterior pituitary hormones (prolactin, thyrotropin, or luteinizing hormone (lutein) or on plasma cortisol [a reflection of adrenocorticotropin hormone (corticotropin) secretion], or on eight enteropancreatic hormones. A full dose–response study was carried out by administering eight single intravenous bolus doses of GHRH to normal men (28, 29). Doses of 0.1 to 10 μg/kg significantly stimulated GH release; the lower doses of 0.1, 0.3, 1.0, and 1.0 μg/kg resulted in more prolonged stimulation of GH release and a second “peak,” indicative of a biphasic response (Figure 2). Serum insulin-like growth factor I concentrations also increased. The only

notable side effect was transient (<5 min) flushing when the highest dose (10 μg/kg) was administered; there was no evidence of toxicity (28). Similar results were obtained by Rosenthal et al. (30), who used the 44-amino-acid peptide. In addition, GHRH given intravenously every 3 h for 108 h to GH-deficient adults stimulated GH release; this suggested that the GH deficiency was a result of a defect in hypothalamic GHRH secretion and not a somatotroph abnormality (31).
Pharmacokinetic studies included determination of the serum half-life \( (t_{1/2}) \) and metabolic clearance rate of GHRH after a single intravenous injection and after a continuous GHRH infusion. Immunoreactive GHRH was measured by a radioimmunoassay developed by Frohman et al. (32). In their study, single intravenous injections were used, three different doses of GHRH being administered on separate occasions to normal volunteers, and GHRH concentrations were measured over the subsequent 180 min. Bi-exponential analysis revealed that the rate of disappearance from plasma was divided into two linear phases: an initial equilibration phase with \( t_{1/2} \) of 7.6 (SE 1.2) min and an elimination phase with \( t_{1/2} \) of 51.8 (SE 5.4) min. The latter was similar to the disappearance rate of 41.3 (SE 3.0) min observed after cessation of the constant infusion. The calculated metabolic clearance rate (L per m\(^2\) of body surface per day) was 194 (SE 17.5) in the intravenous single bolus study and 202 (SE 16) in the constant-infusion study; these results were not significantly different from one another (32). Subsequent "high-performance" liquid chromatography studies by Frohman et al. (33) demonstrated that the \( t_{1/2} \) was 6.8 min. They determined that rapid degradation and modification of the amino terminus results in cleavage of the first two amino acids, with the remaining fragment being immunologically active. This accounts for the findings in their previous study (32).

Because GHRH is a potential therapeutic agent, studies have been undertaken to determine its effectiveness when administered subcutaneously or intranasally. Normal male volunteers were given different subcutaneous and intranasal doses of GHRH-40, and the results were compared with the response to intravenous GHRH. Both subcutaneous and intranasal administration of GHRH stimulated GH release, but a 30-fold higher subcutaneous dose and a 300-fold higher intranasal dose were required to stimulate an amount of GH release comparable with that after intravenous administration of GHRH (29). The concentrations of immunoreactive GHRH in plasma were 60- and 500-fold higher after intravenous administration than after subcutaneous and intranasal administration, respectively (29). Thus, although the subcutaneous and intranasal routes of GHRH administration stimulate GH release, much larger doses of peptide are required. Because synthesis of GHRH-40 is time-consuming and expensive, it is not currently being administered therapeutically by the intranasal route. The 44-amino-acid form is similarly difficult to synthesize.

As noted above, the active portion of the GHRH molecule resides in the first 29 amino acid residues. Thus, synthesis of shorter analogs is easier and less expensive. Two 29-amino-acid residue GHRH analogs have been administered to humans, and both preparations stimulate GH release. A full dose–response study of [Nle\(^{27}\)]GHRH(1–29)-NH\(_2\) was performed by administering the analog intravenously, subcutaneously, and intranasally to normal men. This analog has one amino acid substitution at position 27 and stimulates GH release in a dose-dependent fashion. Similar to the studies with GHRH-40, a 10-fold higher subcutaneous dose than intravenous dose was required to stimulate a comparable amount of GH secretion, and a 30-fold higher intranasal dose was required to stimulate approximately one-fifth the amount of GH release. This analog was not longer acting than native GHRH (34), and, although it effectively stimulated GH secretion, it is not currently being used for therapy.

Another important physiological question addressed by the early GHRH studies in normal men concerned the similarity of the somatotroph to the gonadotroph in its responsiveness to prolonged stimulation by its hypothalamic-releasing hormone. When GnRH is administered intermittently, luteinizing hormone and follicle-stimulating hormone (follicitropin) are released; when GHRH is administered continuously, however, secretion of luteinizing hormone and follicle-stimulating hormone diminish and cease, suggesting a "down regulation" of the gonadotroph receptor. This observation is the basis for the treatment of precocious puberty or carcinoma of the prostate with long-acting GHRH analogs or agonists, because they provide a reversible "medical gonadectomy" (35–37). Thus, before a suitable treatment regimen could be designed for GH-deficient children, it was necessary to determine whether the somatotroph was responsive to both intermittent and continuous stimulation by GHRH. Normal men were given continuous intravenous infusions of GHRH for 6 or 24 h, and their response to a supramaximal bolus injection at the end of the infusions was measured. GH secretion was preserved during continuous infusion of GHRH, as was the response to the bolus injection of GHRH (Figure 3). These findings suggest that the somatotroph can respond to prolonged stimulation and that treatment with a long-acting GHRH analog may be feasible (38, 39). These findings were confirmed by administering a continuous GHRH infusion to normal men for 14 days. On the 14th infusion day, GH secretion had increased to double the amount measured on the pretreatment day (40).

GHRH as a Diagnostic Agent

In an attempt to define better the abnormality, hypothalamic peptides are frequently administered to patients with suspected hypothalamic–pituitary dysfunction. Administration of thyrotropin-releasing hormone with subsequent measurement of the thyrotropin response, probably the most widely used of these tests, is used to diagnose subclinical hypothyroidism or hyperthyroidism. Repeated administration of GnRH usually is necessary to determine whether functional gonadotrophs are present. Similarly, GHRH has been administered to GH-deficient patients to

![Fig. 3. Mean serum GH, ng/mL (y-axis), during a 24-h placebo (vehicle) and GHRH-40 (hpGRF-40) infusion in normal men. During continuous GHRH stimulation GH secretion is preserved, and there is augmentation of naturally occurring GH pulses. Reprinted, with permission, from ref. 38](image)
determine if functional somatotrophs are present. Although a single intravenous dose of GHRH may stimulate GH release in some patients with idiopathic GH deficiency, repetitive intermittent administration may be necessary to "prime" the somatotroph before significant GH stimulation occurs (31).

Children with short stature of various etiologies have been given GHRH. Children with short stature secondary to intra-uterine growth retardation or with familial short stature had a normal GH response, whereas the majority of those with organic hypopituitarism or idiopathic GH deficiency had a smaller GH response than did children in the other groups; nonetheless, an increase in GH indicated the presence of functional somatotrophs (41). Similarly, four patients with GH deficiency secondary to hypothalamic tumors had a GH response to GHRH (42). These studies indicate that GH deficiency most commonly results from a hypothalamic GHRH deficiency. Thus, children with suspected GH deficiency may now be evaluated with indirect stimuli, such as the classic insulin-induced hypoglycemia or oral l-dopa and intravenous arginine, and with direct pituitary stimulation (i.e., by the GHRH test). A GH response to GHRH may also be used to assess the feasibility of therapy with GHRH. One important caveat is that the degree of GH responsiveness varies in normal subjects and in GH-deficient patients alike. Thus, although most normal subjects have an increase in serum GH of >10 μg/L after an intravenous GHRH dose of 1 μg/kg, a less dramatic increase may not be abnormal (28, 30).

Acromegaly, a disease of excess GH secretion, is most commonly caused by a pituitary adenoma. Rare cases of acromegaly result from GHRH secretion by an ectopic source, such as a pancreatic tumor or carcinoid tumor that stimulates pituitary GH secretion. The prevalence of ectopic GHRH secretion is low. In one survey of 177 unselected acromegalics (25), none had an increase in plasma GHRH, but three other patients with known ectopic GHRH-secreting tumors all had increased plasma GHRH concentrations (25). Thus, the prevalence of ectopic GHRH secretion as the cause of acromegaly is <1%. Measuring GHRH in plasma before any therapeutic intervention can identify ectopic GHRH secretion as the etiology of the acromegaly, and proper attention can be directed to identifying the source. Measurement of GHRH in plasma should be an essential part of the evaluation of a patient with acromegaly.

Acromegalic patients also have been administered GHRH to determine pituitary GH responsiveness. Gelato et al. (43) administered GHRH to 29 acromegalic patients, all but four of whom showed a GH response; the unresponsive patients had received prior pituitary irradiation. Although it is not known whether testing with GHRH is useful in determining the pathophysiology of acromegaly, this test may be useful in assessing the effectiveness of therapies, including pituitary irradiation and surgery. Studies are in progress to determine whether or not the absence of a GH response to GHRH after pituitary surgery is predictive of "cure."

Therapy with GHRH

The traditional treatment of GH-deficient children is administration of growth hormone. Because of the development of Creutzfeldt-Jakob disease in a few young adults who received GH extracted from virus-contaminated human pituitary glands (44), these biological preparations are no longer available in the United States. Fortunately, the development of techniques for biosynthesis of GH by recombinant DNA technology has provided an ample supply of human GH for treatment of these children. Clinical trials of a methionyl-GH and pure GH, currently under way, indicate that these preparations are effective in promoting linear growth and are not associated with toxicity (45, 46).

An alternative treatment for GH deficiency is administration of GHRH to stimulate GH release. GH-deficient children have been treated with GHRH-40, GHRH-44, and a 29-amino-acid GHRH analog, with somewhat inconsistent results (47-52). Despite variations in GHRH preparations, total daily dose, frequency of administration, and total treatment duration among studies, a substantial proportion of children showed increased growth velocity during therapy with GHRH.

In one study of 24 GH-deficient children treated for six months or longer with GHRH-40, 21 had acceleration of growth. The peptide was administered either every 3 h for 24 h, every 3 h overnight only, or by twice-daily subcutaneous injections. Among these three subgroups, regression analysis revealed a correlation between average daily dose and growth velocity; i.e., those children who received the higher total daily dose had the most pronounced acceleration of growth (Figure 4) (49). In two studies with GHRH-44, five of six and five of seven children had an increase in growth rate (50, 51). However, the pretreatment response of serum GH to intravenous GHRH, the concentrations of insulin-like growth factor I in serum, and the maximal GH response during subcutaneous GHRH therapy were not predictors of clinical response (51). An amidated 29-amino-acid GHRH analog was administered to 18 GH-deficient children for at least six months. Twice-daily subcutaneous administration resulted in an increase in height in 12, and eight were classified as "responders" as judged by an increase in height velocity of >2 cm/year (52). The bioavailability of this 29-amino-acid analog is not known. Antibodies to GHRH developed in children receiving all three GHRH preparations; antibodies disappeared in some, decreased in titer in some, and remained unchanged in others during continued treatment or after discontinuation (49, 50, 52). In all studies, the presence of anti-GHRH antibodies did not appear to interfere with the increase in growth velocity. Because there are no parallel studies comparing the growth-promoting effects of GHRH vs recombinant DNA-produced human GH, the possible superiority of one
treatment over the other is not known. However, in seven children who were treated with GH before GHRH treatment, the rates of height increase were similar during both treatment regimens (29). Comparative studies of these two treatments are in progress.

Summary
Study of the physiology of GH secretion entered a new era with the discovery of GHRH. Less than four decades elapsed between the original hypothesis of the existence of hypothalamic-releasing hormones and the characterization of the last of these hormones. The collaboration of many investigators has produced a large volume of information on the regulation of GH secretion by GHRH and other factors and ultimately has led to the therapeutic use of GHRH in the treatment of GH-deficient children.

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