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Growth Hormone Secretagogues as Diagnostic Tools in Disease States

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One of the most active topics in the growth hormone-IGF-1 field is that of the so-called growth hormone secretagogues (GHS). At a time when the isolation of GHRH had not occurred, the GHS were developed as artificial tools to release GH. The interest in these groups of compounds was rekindled when it was realized that they were not surrogates of GHRH nor were they acting through the modulation of the release of either GHRH or somatostatin. With the subsequent cloning of the specific receptor of GHS, and today of the natural ligand for that receptor, named ghrelin, it soon become clear that GHS and the GHS-receptor were part of a new physiological system involved in GH regulation. The dual control of GH secretion became a trinity. GHS releases GH when administered by any route—oral, iv, sc, and even transdermally—with a surprising potency and reproductivity. In addition, GHS when administered together with GHRH exert a synergistic action on GH secretion and that combined administration is the most potent GH releaser to date. Clinical studies have demonstrated that the GHS–GHRH administration may be considered the new "gold standard" test of GH reserve in humans, as the GH secretion so elicited is not altered by gender, adiposity, or age. The combined administration of GHRH plus GHS is able to discriminate between healthy subjects and patients with adult GH deficiency, suggesting a considerable utility in the clinical setting.

Key Words: Growth hormone secretagogues as diagnostic tools.

Introduction

In 1975, investigation into the current family of the so-called growth hormone secretagogues (GHSs) got under way *(1)*. At that time, the isolation of endogenous growth hormone–releasing hormone (GHRH) was so elusive that Cyril Bowers and coworkers developed a new strategic approach of introducing chemical modifications into the enkephalin structure and testing for *in vitro* growth hormone (GH) secretion. With this approach, the first nonopioid peptides with GH-releasing capability were developed, although they were not active in vivo. The data obtained from complex conformational energy calculations were used to relate structural features to the tested GH-releasing capability of these compounds. This approach led to the development, in 1980, of the first highly potent GH-releasing hexapeptide, called growth hormone–releasing peptide-6 (GHRP-6) *(1)*. GHRP-6 specifically releases GH in all species tested so far, including humans. Currently, GHRP-6 is the "gold standard" that all the GHS can be compared with *(2)*, and it is the first milestone in the field of the GHSs (**Fig. 1**).

With the isolation of endogenous GHRH in 1982, interest in GHS faded. However, there was a slowly increasing interest in these compounds when it was subsequently communicated that they operated through non-GHRH receptors and exerted a powerful synergism when administered in addition to GHRH, facts proving that GHS were not artificial surrogates of the endogenous GHRH *(3,4)*. In the ensuing years, the intracellular signaling mechanisms were clarified *(5)*, and a wealth of direct and indirect evidence indicated that GHSs did not operate through GHRH. Finally, interest in the field was bolstered in 1993 when the first nonpeptidyl compound was found (**Fig. 1**) *(6)*. The introduction of hexarelin, a GHRP-6 analogue, led to the testing of GHS compounds in different clinical settings *(7)*. The second milestone in the field was the cloning of the receptor for these nonclassic GH releasers *(8)*. With that accomplishment, researchers in the field were faced with a strange situation: they used very active compounds, which activate a well-characterized receptor, but the endogenous ligand of that receptor was unknown.

The final milestone in the field, to date, has been the isolation of the endogenous ligand of the GHS-receptor (GHS-R). Using the transfected GHS-R as a bioassay,

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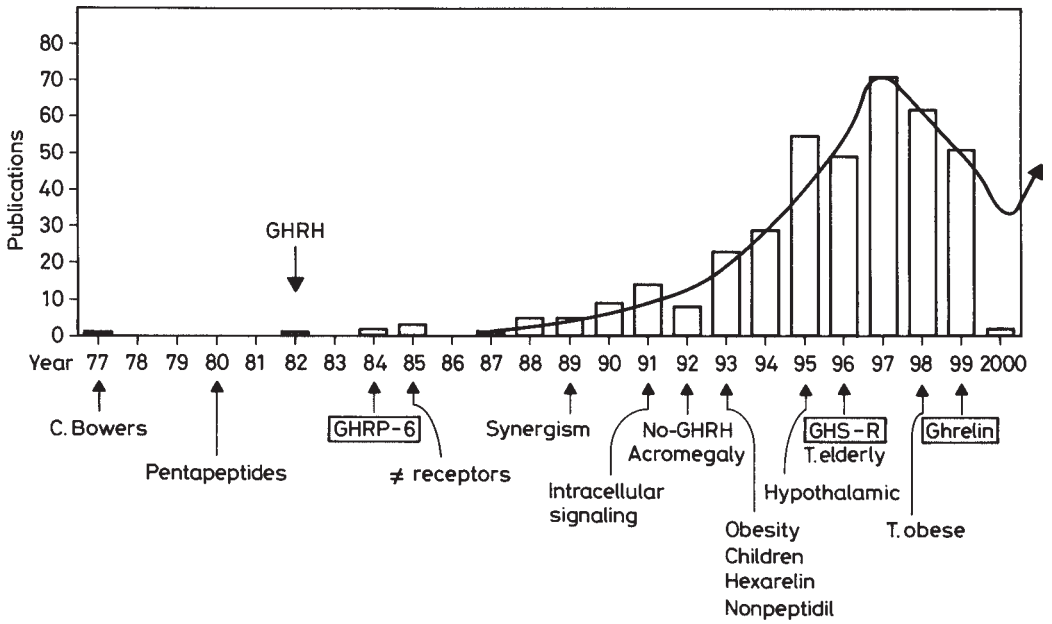

Fig. 1. Number of publications on GHSs per year. GHRH and the GHSs activate different receptors (≠receptors) and exert a profound synergistic activity when administered together. T-elderly and T-obese indicate treatment trials on such groups. GHS-R, receptor for GHSs. In the boxes appear the three more important milestones in the field.

Kojima et al. (*9*) were able to find a 28 amino acid peptide with a high affinity for the receptor, and most surprisingly, the highest concentration was obtained from stomach extracts (*9*). This peptide, now called ghrelin (*ghre* is the Proto-Indo-European root of the word *growth*), which specifically releases GH both in vivo and in vitro, presents new chemical characteristics, such as an *O*-*n*-octanoylation, and its gene is expressed in both the stomach and the hypothalamic arcuate nucleus (*9*). If ghrelin is, as expected, the missing piece of the puzzle, our understanding of the normal and pathologic regulation of GH secretion will increase by leaps and bounds in the coming years and, in parallel, will expand the diagnostic and therapeutic application of natural and artificial GHSs.

The presence of potent artificial GHSs, able to release GH in all species acting via intravenously, subcutaneously, intramuscularly, per os, nasally, and transdermally; the cloning of the GHS-R; and, finally, the cloning of ghrelin are the three pillars demonstrating that GHs are a new physiologic system implicated in GH regulation (**Fig. 2**).

GHSs in Disease States

One of the most interesting properties of GHSs is their ability to act synergistically with GHRH to elicit GH secretion. In fact, when administered together at saturating doses, GHRH+GHRP-6 appears as the most potent stimulus for GH secretion. When this combined test was studied in a classic situation of impeded GH release—obesity—it was observed that obese subjects elicited a massive discharge of GH *(10)*. This surprising finding indicates that the absence of GH secretion in obese individuals is a functional and reversible problem, and that somatotroph cells in pituitaries of obese subjects may be blocked for years but suddenly be able to respond after the appropriate stimulus. A similar finding was observed in aging, a physiologic state associated with diminished GH secretion *(11)*. When normal old subjects, ranging from 75 to 96 yr, were tested with GHRH+GHRP-6, the GH secretion was very important and identical to that presented by young (19–40 yr) or middle-aged (46–65 yr) individuals (*12*). These observations opened up the way for trials for verifying whether GHSs may be of utility in the treatment of either obese patients under a hypocalorie diet or aged subjects, as well as patients under negative energy balance *(13–15)*.

Patients with acromegaly show an enhanced secretion of GH when stimulated with GHRH, GHSs, or a combination of GHRH+GHRP-6; however, the synergistic action is not observed in these patients, suggesting that part of the hypothalamic mediation in the GHS activity is not operative *(16)*. On the other hand, in patients with prolactin-secreting pituitary macroadenomas, the GHRH+GHRP-6-mediated GH secretion is impeded. This impairment must be explained by a functional blockade of the hypothalamopituitary connection, because when the adenomas shrink owing to the dopaminergic analog treatment, the GH secretion after the combined stimulus is restored *(17)*. Although in some situations of hypercortisolism the GH secretion after the administration of GHSs is preserved, this may be a time- and dose-dependent phenomenon. In chronic situations of hypercortisolism, such as in Cushing syndrome, the GH release after the combined challenge appears to be severely

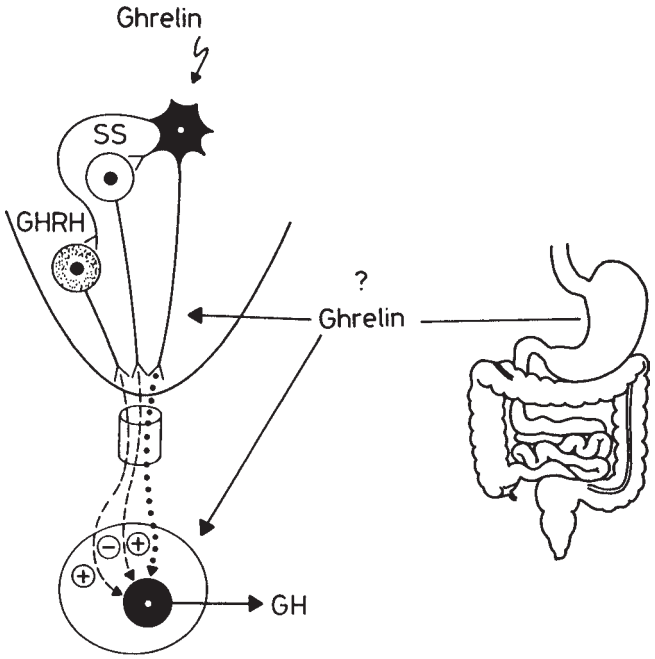

Fig. 2. A new physiologic system involved in the regulation of GH secretion, involving GHRH, somatostatin (SS), and ghrelin.

blocked *(18)*. The basic mechanisms involved in the severe impairment of GH secretion observed in chronic hypercortisolism are unknown at present, but corticoids are well known as agents able to disturb the basic cell transcription mechanisms and signaling systems, and these perturbatory actions may last for several months or years *(19)*.

Although GHSs were developed as activators of pituitary somatotrophs and the iterative structural modifications were undertaken using pituitary cells in vitro, considerable evidence was gathered suggesting that these compounds may have relevant hypothalamic actions. In fact, receptors for GHSs were detected in pituitary and hypothalamus, the synergistic action of GHRH and GHRP-6 is observed in vivo but not in vitro, and in the rat GHS behave like a hypothalamic neurohormone (*20*). A further demonstration of the hypothalamic actions of these compounds is that GHSs administration enhances c-fos expression in hypothalamic neurons (*21*). This indirect evidence was confirmed when it was demonstrated that to be fully operative, GHSs require the presence of a functional hypothalamus. In patients with intact pituitaries, but with a tumoral mass leading to a hypothalamopituitary disconnection (functional stalk section), the release of GH after the administration of GHRH was preserved whereas that after hypoglycemia was absent. In these selected patients, the GH-releasing capability of GHSs and GHRH+GHRP-6 disappears, demonstrating that the action of GHSs at the hypothalamic level is crucial, whereas the pituitary one is ancillary *(22)*. These findings have been confirmed in children with hypothalamopituitary disconnection owing to neonatal stalk transection *(23)*.

GHSs as Diagnostic Tools

Considering that GHSs are highly effective stimulators of GH secretion and that the combined administration of GHRH+GHRP-6 is now the most powerful stimulus of GH secretion *(24)*, the possible utility of this combined administration as a provocative test of GH reserve has been extensively studied. This combined test possesses some invaluable characteristics that give it certain advantages when faced with the classic insulin tolerance test (ITT) or hypoglycemia test. For example, the GHRH+GHRP-6 test is not affected by the metabolic status of the subjects under testing, nor by the glucocorticoid, thyroid, or gonadal steroid levels of the subject, thus, does not require a perfect hormone replacement therapy in the hypopituitary patients under testing *(25)*. The test is not affected by the presence of concomitant diabetes mellitus, and there are no contraindications *(26)*. The utility of the combined GHRH+GHRP-6 test as an alternative to ITT in the diagnosis of GH deficiency in adults is enhanced when considering that the combined test evaluates the whole hypothalamopituitary axis and not only the pituitary, it is not affected by age, and it is not affected by adiposity. In fact, when studying adults with GH deficiency, age and adiposity are a common confounding factor, and because several of the subjects under testing suffered from neurosurgical interventions, a test devoid of both side effects and contraindications is mandatory *(27)*.

The combined test is currently under evaluation in our laboratory in a large group of both control subjects and GH-deficient patients. However, before evaluating the utility of the test in sorting the healthy from diseased, the behavior of the test itself was assessed in order to study its reproducibil-

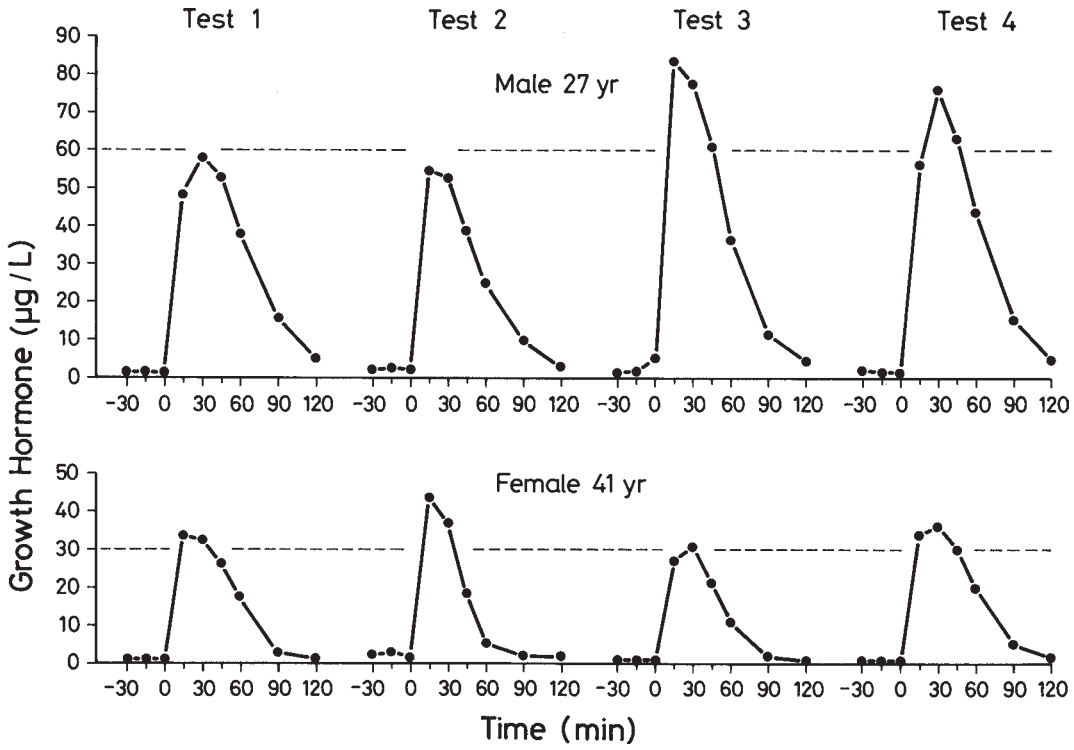

Fig. 3. GH secretion elicited in two normal subjects after the administration of 1 µg/kg of GHRH plus 1 µg/kg of GHRP-6 iv on four different occasions separated by at least 1 mo.

ity. When a large group of healthy subjects was assessed using the combined test four times on separate days at least 1 mo apart, the reproducibility was outstanding (**Fig. 3**). Although minor variability was observed among the four different tests, both as a maximal peak or as area under the secretory curve, the coefficient of variation was low and the regression analysis good and significant (data not shown). The combined GHRH+GHRP-6 test not only has the best reproducibility of the different provocative tests of GH reserve, but probably is the only one that can be subjected to extensive evaluation. Other tests, such as the ITT have unpleasant effects and potential risk, thus preventing the possibility that the ITT may be evaluated in a large group of normal volunteers or in aged normal subjects. Because it is practically impossible to conduct a study with repeated ITT testing, a thorough, long-term evaluation will be needed. In any case, the partial studies performed in evaluating the ITT have challenged the reproducibility of that test *(28–30)*.

GH-deficient patients diagnosed with severe GH deficiency by a response to ITT lower than 3 µg/L and sex and age-matched normal subjects have been evaluated in our laboratory using the combined GHRH+GHRP-6 test as a provocative test of GH reserve. In control subjects, GH secretion after the test was not affected by age and scarcely affected by adiposity, and in GH-deficient patients, the GH peak was unaffected by age, adiposity, and insulin-like growth factor-1 basal levels, thus proving in large series previous experimental findings. When the GH response to the combined stimulus was correlated with that elicited by ITT, a significant correlation, but with a poor *R* value, was obtained, suggesting that both tests are evaluating similar but not identical altered mechanisms (data not shown). The preliminary data suggest that the GHRH+GHRP-6 test is at least as good as the ITT, and probably better in sorting healthy from diseased subjects, with the added value of being devoid of risks and side effects.

Conclusion

In the last 20 yr, we have observed and participated in the development of a new area of endocrinology, that of the so-called GHSs. This fascinating history that started when Bowers and coworkers invented, not discovered, these nonclassic peptides has culminated with the cloning of the GHS-R and ghrelin. Ghrelin is probably the endogenous ligand for the receptor, although it is not proven that there is only one receptor or only one ligand. In the next few years, we foresee the rapid growth of knowledge in the basic mechanisms that regulate GH secretion in healthy and diseased subjects, as well as in the therapeutic and diagnostic uses of both natural and artificial GHSs.

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