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Current Topics in GH Secretagogue Research—Introduction

Excerpts from the Fourth International Symposium on GH Secretagogues Clearwater, FL (November 7–11, 2002)

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The field of growth hormone (GH) secretagogue research evolved in two separate directions. This divergence, which ultimately lead to discovery of uniquely different classes of molecules capable of regulating GH release by separate mechanisms, originated from a common goal. That goal, which was to better understand how the brain regulates pituitary function, ultimately contributed to our current understanding of the field of neuroendocrinology. The search to identify the link between the nervous and endocrine systems intensified during the late 1960s and early 1970s when researchers were vying for the honor of being the first to describe the molecular structure of the hypothalamic neuropeptides. A classic approach to this problem was to extract and test fractions of the hypothalamic homogenates for their ability to affect synthesis and release of hormones from pituitary tissues. However, a significant obstacle to these efforts, especially for identification of a GH-releasing molecule, was lack of sufficient material to facilitate sequencing the peptides by the technology of the day. Eventually, however, it was discovered that some patients with pancreatic tumors displayed characteristics of acromegaly. It was correctly assumed that this clinical presentation resulted from exposure to a GH-releasing hormone (GHRH) that might be the same as the one produced by the brain to regulate pituitary production and release of somatotropin. The advantage of having the tumor tissue was that it produced sufficient material for sequencing as well as for functional analysis of the molecule. Eventually, the peptide produced by the tumors and brain was found to be the same, resulting in simultaneous publication of the structure for GHRH by Drs. Guillemin and Vale. As a consequence of this and other new information on neuro/endocrine functions, Guillemin and Schally shared the Nobel Prize for Physiology in 1977 for their work on hypothalamic peptides.

During the time that Guillemin and Vale were taking a traditional approach to the discovery and characterization of hypothalamic GHRH, another research team headed by Dr. Cyril Bowers was using a more novel approach to the problem. Recognizing the limitations of working with hypothalamic tissues while assuming that there might be a family of hypothalamic peptides with similarities in structure, Bowers' team modified some of those peptides whose structures were known and screened them for bioactivity. They seemed to be successful in proving this hypothesis when a derivative of enkephalin proved to have weak GH-releasing activity in vitro. The prototype molecule was a hexapeptide, His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2, which was subsequently named GH-releasing hexapeptide or GHRP-6. Further work with the molecule showed that it potentiated GHRH activity, especially in vivo, and that the molecule worked through specific, saturable receptors and intracellular second messengers that were distinctly different from GHRH *(1)*. The timing of discovery of these co-secretagogues seemed to be fortuitous for clinical application because they occurred early in the 1980s. The practical relevance of this fact is that, in 1983, the limited availability of cadaver-derived GH reached crisis proportions when it was discovered some preparations contained Creutzfeldt–Jakob virus prion. Furthermore, the virus was transmitted to people being treated with the hormone. This significant risk of using GH for promoting growth in children with GH deficiency suggested that GH secretagogues such as GHRH and GHRP might provide alternatives to cadaver-derived GH. However, this potential was overridden by several factors. First of all, they were very expensive to produce by chemical means even though their size was quite small compared to GH. Furthermore, because the primary subjects for GH therapy at the time were GH-deficient children who were unable to produce endogenous GH, there was not much incentive to develop GH secretagogues that required a functional pituitary gland to be effective. Even recently, a study evaluating safety and efficacy of GH secretagogues in short-stature children sponsored by Merck was abandoned because of disappointing growth results in the second 6 mo of treatment. Prior to that failure, Wyeth-Ayerst discontinued a study using the GHRP-6–related xenobiotic, GHRP-2, also because of poor efficacy and problems of compliance in that the compound had to be administered two or three times daily after fasting for an extended period of time. As a result of these commercial

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failures, the major incentive for development of GH secretagogues would come later when hormone replacement therapy was identified as a potentially useful approach for sustaining health and vitality in aging persons whose pituitary functions were waning because of advancing age.

Work on these xenobiotic GH-releasing peptides and nonpeptidyl analogs accelerated in the 1990s after publication of data showing that GH administration to older, but otherwise healthy, men increased muscle mass and decreased abdominal adiposity *(2)*. This singular report stimulated emergence of a new "field" of medicine called "anti-aging," the objective of which was purported to be the maintenance of health and vitality during the latter stages of life. The reason to investigate hGH administration to elderly subjects was based at least in part on the observation that the phenotype of an aging human being resembles, more or less, that of a younger adult afflicted with GHD. In other words, it is the similarities in body composition of old persons and GH-deficient adults that provided the basis for the hypothesis that decreased endogenous GH secretion may contribute to some of the age-related changes in body composition *(3)*. Other factors included the fact that GH-deficient children who did not continue to receive GH as adults or those that became GH deficient as adults suffered greater morbidity and mortality than the general population. Also, diseases of GH deficiency are many of those associated with aging. Conversely, GH replacement reversed some of those disease symptoms, and, because GH is one of the first hormones to decline during aging in association with increased morbidity and mortality, it seemed reasonable that progressive loss of the hormone could contribute to the process of senescence. Accordingly and almost immediately following publication of Rudman's data *(2)*, GH replacement was proposed as an "anti-aging therapy" by entrepreneurs who correctly predicted a huge public demand for such treatment. Especially important for business purposes was the fact that large numbers of the affluent "baby boomer" generation were passing beyond middle age, becoming acutely aware of their mortality and were prepared to purchase the rejuvenating hormone, no matter the cost.

As a result of this interest and the commercial potential therein, nonpeptidyl mimics of growth-hormone-releasing peptide were developed by the pharmaceutical industry. The new molecules were orally active and readily bioavailable *(4)*. Significantly reduced cost of goods for nonpeptidyl mimics of GHRP and for GHRH produced by gene recombination technology made GH secretagogues important alternatives to recombinant GH for use in aging. Besides cost considerations, GH secretagogues offered different effects on the GH neuroendocrine axis that provided the clinician with treatment choices not available with GH alone. A rapidly growing base of information on the efficacy of these molecules suggested that they were potentially co-secretagogues of GHRH and the search for an endogenous ligand was initiated. This goal was achieve in 1999, resulting in the identification of a 28-amino-acid molecule. Interestingly, it was produced by the gut and, as such, was reminiscent of the fact that GHRH was also originally described in peripheral tissue. The endogenous compound was named ghrelin *(5)*.

One of the most important differences between GH and GH secretagogues is the loci of their respective actions. The primary site of GH action is the liver, whereas that of GH secretagogues is the pituitary gland. Secondary loci of action, especially for the xenobiotics of the ghrelin type, have also been reported in the heart, brain, and gut. Although binding sites that are specific for GH secretagogues are distributed throughout the body, their localization in the heart is striking *(6–10)*. The distinctive characteristic of specific cardiac binding of such secretagogues as ^{125}I-Tyr-Ala-hexarelin is that it is often higher than that in the pituitary *(6,8,9)*. These binding sites apparently have functional properties because prolonged treatment with peptidyl secretagogues protects against cardiovascular damage in aged rats and also in GH-deficient rats with post-ischemic ventricular dysfunction *(11–13)*. They also improve cardiac performance after myocardial infarction *(14)*, protect against diastolic dysfunction of myocardial stunning in isolated, perfused rabbit heart *(15)*, and enhance left ventricular contractility in animals with heart failure *(16)*. Whether these effects can be demonstrated for the natural GH secretagogues ligand, ghrelin, remains to be determined. In addition to these few effects, a multitude of other actions on the heart have been amply reviewed (see ref. *10*).

Central effects of GH secretagogues include their orexigenic and sleep effects. Administration of synthetic and endogenous secretagogues induce remarkable weight gain in animals by increasing food intake and reducing fat utilization *(17–26)*. These activities are not related to the anabolic effects of GH and are probably mediated by neural networks modulated by leptin *(17–25)*. This view is supported by the fact that GH secretagogue analogs that are devoid of any GH-releasing activity sometimes stimulate food intake *(23,24,27)*. With regard to sleep, oral MK677, a synthetic GH secretagogue, has been reported to increase REM sleep while decreasing its latency *(28)*. Peptidyl secretagogues also modify sleep patterns in normal subjects suggesting that a potential application for these compounds in aged subjects would be to rejuvenate healthy and restful sleep. Finally, preliminary reports of a tumor-inhibiting effect of some GH secretagogues are of great potential interest in aging, and the implications for their use in anti-aging medicine are obvious (see ref. *10*). A summary of the endocrine and nonendocrine actions of ghrelin and its synthetic analogs are presented in Table 1.

So in review of these differences between GH and GH secretagogues, it is relevant to note that differences in specific properties, binding loci, and mechanisms of action

Table 1
Specific Activities Attributable to GH Secretagogues

Endocrine Related
GH releasing
Prolactin releasing
ACTH releasing
CNS Related
Sleep inducing
Orexigenic
Soma Related
Gastric motility and acid release
Modulation of pancreatic activity and glucose metabolism
Cardiovascular activity
Antiproliferative activity

Table 2
Characteristics of GH and GH Secretagogues

	rGH	Secretagogues
Production	Recombinant gene technology	Chemical synthesis
Source	*E. coli* or mouse cell	N/A
Viral potential	None	None
Target tissue	Liver	Pituitary (heart, brain, gut)
Time for tissue exposure	Instantaneous (GH injected directly; exogenous)	Delayed (GH secreted from pituitary after injection; endogenous)
Duration of tissue exposure	Long (square wave)	Short (episodic release)
Efficacy	More rapid and pronounced; clinical effects more dramatic	Less rapid and pronounced; less clinical effect
Type	Pharmacological	Physiological
Effect on pituitary	Shuts down endogenous production and secretion	Stimulates production and secretion
Toxicity potential	Greater, but dose dependent; little at doses in protocol	Very little if any
Ancillary effects	Direct on bone; may have benefit in relieving joint pain	May facilitate natural sleep, orexigenic

relate to the clinical applications for these compounds. As previously stated, GH secretagogues are not suited for conditions in which the pituitary gland is destroyed or irreparably dysfunctional, as in certain types of GHD patients. Furthermore, because GH secretagogues stimulate the pituitary gland to produce and release GH, one cannot reliably quantify the amount of GH exposure resulting from their use. In other words, unlike GH, which can be administered at a specific dosage such as 10 µg/ks bw, it is impossible to quantify GH exposure following administration of GH secretagogues. This is especially true over time, because pituitary priming increases GH mRNA and pituitary GH content upon repeated use. However, this effect is not necessarily bad, especially when secretagogues are used in aging subjects. The reason for this view is that administration of the GH-releasing compounds seems to rejuvenate the pituitary, increasing activity of the somatotroph phenotype and decreasing that of the lactotroph phenotype *(29)*. This effect tends to reduce age-related hyperprolactinemia and also may be useful for treating lactotroph adenomas.

Another important action of GH secretagogues and/or GH includes that on the immune system. Hormone administration causes thymic recrudescence in rats and mice and also significantly changes T cell phenotypes *(30)*. Similar effects have been seen in human subjects, but it is still not clear whether these effects represent a positive effect on the immune system that would be of benefit to delaying or reducing the maladaptive effects of aging on health and vitality. A summary of the differences between GH and GH secretagogues is presented in Table 2.

Over the course of the past decade, we have periodically sponsored International Symposia on GH secretagogues in an attempt to maintain a forum for advances in this very important field of neuroendocrinology. Those meetings included the following:

International Symposium on Growth Hormone Releasing Secretagogues, St. Petersburg, FL, 1994 (Serono Symposia; Chairpersons, B.B. Bercu, R.F.Walker)

Workshop Conference on Growth Hormone Releasing Secretagogues, US Food and Drug Administration, Bethesda, MD, 1994 (Chairpersons, B.B. Bercu, R. F. Walker)

Second International Symposium on Growth Hormone Releasing Secretagogues, Tampa, FL, 1997 (Chairpersons, B.B. Bercu, R.F. Walker)

Third International Symposium on Growth Hormone Secretagogues, Keystone, CO, 2000 (Chairpersons, B.B. Bercu, R. F. Walker)

Fourth International Symposium on Growth Hormone Secretagogues, Clearwater, FL, 2002 (Chairpersons, B.B. Bercu, R. F. Walker)

It was during the Third International Symposium that discovery of ghrelin was first reported in public forum. Selected subjects from the Fourth International Symposium that was recently held in Clearwater are published herein.

In conclusion, the value of GH secretagogues as useful research, diagnostic, and therapeutic molecules is clear. Obviously, there are certain limitations to their use as an alternative to GH itself. The most obvious of these is the fact that GH secretagogues are only effective in stimulating production and release of endogenous hormone so that they are not generally effective for treating pathologic GHD (GH-deficient) syndromes. Thus, the most relevant condition for making a choice between one or the other of these compounds for GH replacement therapy may be aging, because in healthy people, the pituitary remains responsive to stimulation or can be induced to respond. Because of this fact, the major advantage of exogenous hormone in children of GHD adults, which is the ability to achieve high and sustained levels of circulating GH, is not so important in the healthy aging population. In fact, it may actually oppose the end-points that are sought in aging persons including long-term reconstitution of stable physiological functions.

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