CMLS, Cell. Mol. Life Sci. 54 (1998) 1316–1329
1420-682X/98/121316-14 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Growth hormone-releasing peptide (GHRP)

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Received 7 April 1998; received after revision 7 August 1998; accepted 7 September 1998

Abstract. Growth hormone-releasing peptides and non-peptides (GHRPs, GHRP-GHS) are a new chemical class of GH secretagogues with a chemistry that ranges from small synthetic peptides to peptidomimetics. They release GH in animals and humans by a unique dual and complementary action on the hypothalamus and pituitary. Although the present GHRPs are of unnatural origin, evidence by a number of investigators is gradually accumulating to support that GHRP reflects the GH-releasing action of a new natural hypothalamic hormone yet to be isolated and identified. Despite the de novo origin of GHRP, a major reason for the persistent investigation is because of the possible practical diagnostic and therapeutic value in humans as well as the potential theoretical value of new insight into the physiological regulation of GH secretion.

Key words. Growth hormone; GHRP; GHRH; SRIF; pituitary; hypothalamus; U-factor.

Introduction

Growth hormone-releasing peptide (GHRP) evolved from a process recently designated by Michael Conn as 'reverse pharmacology' [1]. This aptly indicates the artificial origin of GHRP and underscores the amazement and surprise that the unnatural is gradually and persistently evolving into the natural. Although the synthetic GHRPs being developed were thought to mimic the GH-releasing action of a new natural hypothalamic hormone, it was apparent from the uncoded D-amino acid residues of the unnatural synthetic GHRPs that the amino acid sequence of the presumed natural hormone would be different.

Synthetic GHRPs, which have been developed by several different groups, can be considered as small peptides and peptidomimetics. Although they are all GH secretagogues, in this overview they have been designated as either GHRPs or GHRP-GH secretagogues (GHRP-GHS) in order to generically include the overall group and to distinguish this class of GH secretagogues from the many other types of GH secretagogues that exist. Despite the unexpected wide range in chemistry of the various GHRP-GHSs, most of the present evidence supports that they all act via the same receptor and by the same hypothalamic-pituitary endocrine and molecular mechanism. Nevertheless, findings that are implied by the different chemistries of the GHRP-GHSs include the possibility of receptor subtypes as well as the possibility of more than one intracellular signal transduction pathway.

As might be expected, the GHRP saga has been circuitous, and many talented investigators have been responsible for what has occurred. Because of the briefness of this summary, most of the GHRP studies of other investigators will only briefly be reviewed.

Historical background

In 1976, a series of synthetic enkephalin opiate analogues were studied because the enkephalins were natural small peptides of the brain and because opiates were known to release GH. Thus in some way these natural opiate peptides could have been related to the elusive putative natural growth hormone-releasing hormone (GHRH). Even though opiates usually were considered to release GH via a direct hypothalamic rather than a pituitary action, and were not thought to be GHRH itself, the possibility was considered that these natural peptides might release GH by a dual hypothalamic and pituitary action. For this reason Met and Leu enkephalin as well as their analogues were studied for a direct pituitary action in vitro. Noteworthy was that the pentapeptide $TyrDTrpGlyPheMetNH_2$ ($DTrp^2$), which was related to the native Met enkephalin TyrGlyGlyPheMetCOOH, was found to release GH in vitro, but its potency was low (3–20 μg/ml medium) [2, 3]. However, some select in vitro findings which indicate the importance of this pentapeptide are the following. After many years of unsuccessful attempts to isolate the putative natural GHRH, a small synthetic molecule with a known amino acid sequence was now available to demonstrate the release of GH by a direct pituitary action. $DTrp^2$ had no opiate activity and was specific in action in that it did not release thyrotropin stimulating hormone (TSH), luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin (PRL) or adrenocorticotropin hormone (ACTH). Although somewhat unexpected, $DTrp^2$ did not release GH in vivo. Since it had the presumed direct pituitary action of the putative natural GHRH, it was considered to mimic the action of GHRH and to be a small peptide with activity that could be improved upon by the same structure-activity approach that we utilized between 1969 and 1976 in the development of thyrotropin releasing hormone (TRH) and luteinizing hormone releasing hormone (LHRH) analogues [4, 5].

Between 1978 and 1980 theoretical conformational studies performed by Momany were incorporated, and many GHRP analogues were synthesized. Eventually these new analogues had both in vitro and in vivo activity. As recorded in table 1, during this time period four chemical classes of tetra- and pentapeptide GHRPs were developed, that is, $DTrp^2$, $DTrp^3$, $DTrp^{2,3}$ and $DTrp^2LTrp^4$ [3, 6, 7]. The GHRPs developed before 1980 were only active in vitro. A chemical hallmark of these small synthetic GHRPs is the presence, position, number and stereochemistry of the Trp residues. The first three classes evolved empirically by varying the amino acid composition and modifying the stereochemistry, sequence and length of the peptide and by determining the GH-releasing activity of the peptide in vitro. By the combined empirical-theoretical approach, the first in vitro and in vivo active hexapeptide, $HisDTrpAlaTrpDPheLysNH_2$ (GHRP-6), evolved from the $DTrp^2LTrp^4$ class of GHRPs [8, 9].

Table 1. Classes of GHRPs active only in vitro (1976–1980).

1	$TyrDTrp^2GlyPheMetNH_2$	($DTrp^2$)
2	$TyrAlaDTrp^3PheMetNH_2$	($DTrp^3$)
3	$TyrDTrp^2DTrp^3PheNH_2$	($DTrp^{2,3}$)
4	$TyrDTrp^2AlaTrp^4DPheNH_2$	($DTrp^2LTrp^4$)

Table 2. GHRP-GHRH relationship on GH release.

	GHRP-GHRH relationship	Supported by the results of
A.	Independent	antagonists and cAMP in vitro
	Dependent	GHRH antiserum in vivo
B.	Additive	GHRP + GHRH GH response in vitro
	Synergistic	GHRP + GHRH GH response in vivo
C.	Permissive	pituitary cAMP in vivo

Biological actions

About the same time the isolation of natural GHRH was accomplished in 1982, the following series of bioactive results of GHRP-6 were reported. 'GHRP-6 significantly released GH in vitro from the pituitary of immature female rats at 1 ng/ml incubation medium. The control GH value in ng/ml ± SEM was $-167 + 114$ while the stimulated values were 955 ± 272 ($< .01$) at 1 ng, 1603 ± 305 at 3 ng, 2244 ± 172 ($< .001$) at 10 ng and 2198 ± 358 ($< .001$) at 30 ng. When this peptide was administered to unanesthetized 21 day old female rats, acute release of GH (ng/ml serum) by 1, 10 and 100 μg was 12 ± 4 ($< .02$), 151 ± 56 ($< .02$) and 381 ± 61 ($< .001$), respectively, the control value was 1 ± 0.6. Additionally the peptide significantly ($< .001$) augmented the body weight gain of 16 day old female rats by a net increase of 17.5 and 10% after 9 and 25 days of treatment with 30 and 100 μg once or twice daily. At the end of this treatment period the peptide also was found to release GH acutely after administration of 30 μg. Other results of this GHRP include the following. The GHRP-6 did not release LH, FSH, TSH or PRL in vitro or in vivo (rat). Somatropin releasing inhibiting factor (SRIF) 1-14 and 1-28 inhibited the GHRP stimulated GH release (rat) in vivo and in vitro with SRIF 1-28 being more potent, especially in vivo. GH levels rose acutely 10–25 fold at 2–10 min in rhesus monkeys, lambs, and calves. Also on repeated administration of low and high dosages of GHRP to immature rats, it was possible to demonstrate an equivocal potentiation and an unequivocal down regulation of the GH response' [10].

In 1984, we reported that despite the GHRH-like activity of GHRP, our data suggested that the GHRP action on

GH release involved a different somatotroph receptor from that of GHRH [9]. Between 1982 and 1984, interactions between GHRP, GHRH-29/40/44 and the kappa opiate agonist MRZ-2549 were studied in the rat. Repeated GHRP injections desensitized the peptide's GH release without altering the GH release induced by GHRH. In addition, the maximal GH response to GHRH could be increased by simultaneous GHRP injection. 2549 had a synergistic effect on both the GHRH and GHRP responses. The GH response of both 2549 and GHRP could readily be desensitized. Desensitization by 2549 did not decrease the GHRP or GHRH-GH release. In vitro results supported the in vivo results in that more GH was released in vitro by GHRP and GHRH together than the peptides alone, and during periods of homologous desensitization by GHRP, GHRH was fully active. 2549 was not active in vitro, and thus its in vivo GH-releasing activity is due to an extrapituitary site of action. Collectively the results indicated these three different types of GH secretagogues released GH by different but overlapping and complementary mechanisms. From these results it was concluded 'that indirect evidence suggested that the pituitary somatotroph has multiple agonist receptors. It is possible that several endogenous ligands with GH releasing activity have yet to be identified [11].

Additionally, in 1984, it was postulated that GHRP may reflect the activity of another natural hypothalamic hormone involved in the regulation of GH release [9]. Subsequently, a considerable number of findings by many investigators have continued to support this possibility.

The classical neuroendocrine concept and approach utilized in the establishment of the known hypothalamic hypophysiotropic hormones, that is, TRH, LHRH, GHRH, had to be modified in order to assess and elucidate the full spectrum of actions of GHRP on GH release, because GHRP appeared to act in a complementary way on both the hypothalamus and pituitary to release GH, whereas the classical hypophysiotropic hormones have a direct positive pituitary effect and a negative hypothalamic effect. Until recently, only a very limited number of in vitro studies have been performed directly on the hypothalamus; however, evaluation of the hypothalamic action of GHRP has been assessed more directly in vivo by administration of GHRP intracerebroventricularly and by direct GHRP administration into localized areas of the hypothalamus. In addition, GHRH and SRIF have been measured in hypophyseal portal blood after administration of GHRP. Between 1989 and 1992, three studies revealed specific high-affinity binding sites for GHRP in the pituitary and also the hypothalamus [12–14].

The relationship between the actions of GHRP and GHRH in vitro and in vivo is outlined in table 2. Most

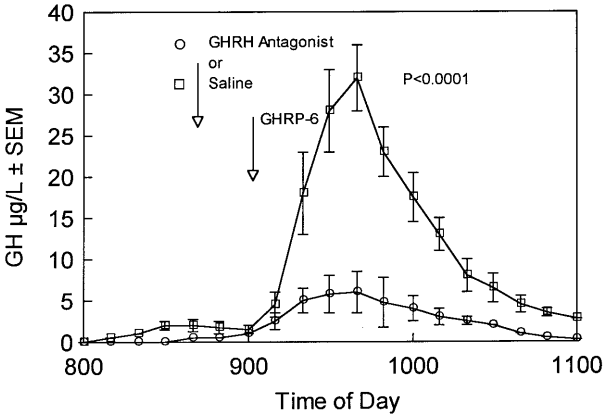

Figure 1. GH responses to GHRP-6 after saline and GHRH antagonist (400 µg/kg) treatment, $n = 9$ [19]. Reprinted with permission from: Pandya N, Demott-Friberg R., Bowers C. Y., Barkan A. L. and Jaffe C. A. (1998) Growth hormone (GH)-releasing peptide-6 requires endogenous hypothalamic GH-releasing hormone for maximal GH stimulation. J. Clin. Endocrinol. Metab. **83:** 1186–1189, © 1998 The Endocrine Society, Bethesda, MD.

results reported over the last several years have indicated the existence of a more unique relationship between GHRP and GHRH than between GHRP and SRIF [15–18]. In earlier studies, the pituitary was considered to be the predominant anatomical site of action of GHRP, but gradually more emphasis has been on the hypothalamus. Even though the hypothalamus is currently considered to be the more predominant anatomical site of action(s), this action is still incompletely understood, whereas the GHRP pituitary action on the phosphoinositol-protein kinase C intracellular pathway is well established and different from the activation of the adenyl cyclase-protein kinase A pathway of GHRH.

A basic important in vivo issue still unresolved is to what degree endogenous GHRH, which may be increased by the hypothalamic action of GHRP, is the direct or major mediator of GH release. Collectively, our in vivo results in animals and humans on the hypothalamic action of GHRP indirectly indicate that at low doses of GHRP endogenous GHRH release is not increased. Nevertheless, the data also indicate that endogenous GHRH plays an essential but only a permissive or passive role in the release of GH induced by lower doses of GHRP rather than being the direct mediator of this release [15–18]. In contrast, at high doses, endogenous GHRH release presumably is increased by GHRP, and thus endogenous GHRH directly plays an active rather than a passive role in the GH released by GHRP. The recent results of Pandya et al. (fig. 1) demonstrate that a GHRH antagonist markedly inhibited the GH response of

GHRP-6 in normal young men [19]. Because of the differences in the in vitro and in vivo actions of GHRP as well as the probable in vivo dose dependency of endogneous GHRH, the in vivo GHRP actions and

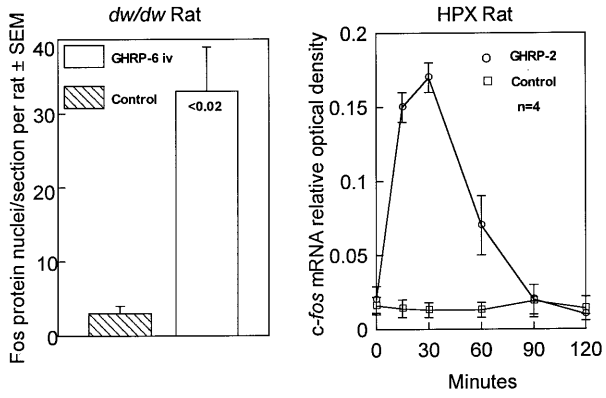

Figure 2. Stimulation of arcuate neurons by GHRP. Specific evidence for the direct hypothalamic action of GHRP in the *dw/dw* rat (left panel) [23]. Reprinted with permission from: Dickson S. L., Doutrelant-Viltart O. and Leng G. (1995) Growth hormone (GH) deficient *dw/dw* rats and *lit/lit* mice show increased *fos* expression in the hypothalamic arcuate nucleus following systemic injection of GH-releasing peptide (GHRP-6). J. Endocrinol. **146:** 519–526, © 1998 The Endocrine Society, Bethesda, MD. Evidence of increased c-*fos* mRNA after GHRP treatment in the hypophysectomized (HPX) rat (right panel) [25]. Kamegai J., Hasegawa O., Minami S., Sugihara H. and Wakabayashi I. (1996) The growth hormone releasing peptide KP-102 induces c*fos* expression in the arcuate nucleus. Mol. Brain Res. **39:** 153–159, © 1998 Elsevier Science.

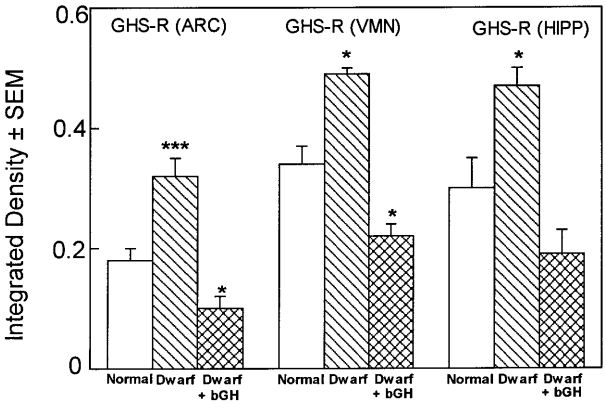

Figure 3. Effect of 200 μg/day bovine GH (hGH) treatment in *dw/dw* female rats for 6 days. $*P < 0.05$, $***P < 0.001$ vs. control group. $n = 5–6$/group [31]. Reprinted with permission from: Bennett P. A., Thomas G. B., Howard A. D., Van der Ploeg L. H. T., Smith R. G. and Robinson I. C. A. F. (1997) Expression and regulation of the growth hormone secretagogue-receptor (GHS-R) gene in normal and dwarf rats. Endocrinology **138:** 4552–4557, © 1998 The Endocrine Society, Bethesda, MD.

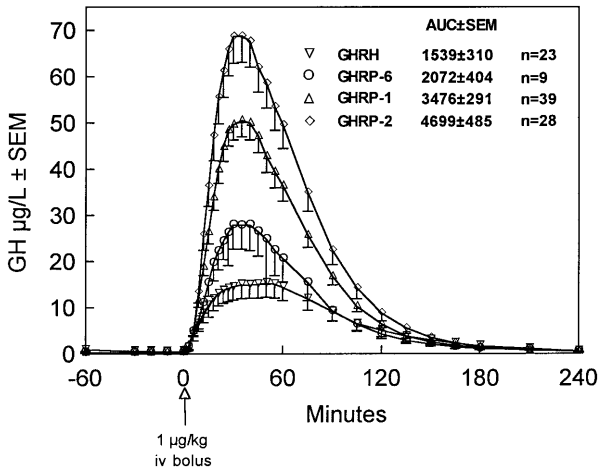

Figure 4. Effect of GHRP-6, GHRP-1, GHRP-2 and GHRH in normal young men. Values are mean ± SEM. AUC, area under the curve [17]. Reprinted with permission from: Bowers C. Y. (1996) Xenobiotic growth hormone secretagogues. In: Growth Hormone Secretagogues, pp. 9–28, Bercu B. and Walker R. (eds), © 1998 Springer, New York.

interrelationships with GHRH are confusing and convoluted. In vitro data on the direct pituitary action of GHRP and GHRH, alone and together, does not explain a number of the observed in vivo GH-releasing actions of these peptides.

A general point about the GHRPs concerns the importance of distinguishing and considering the differences between the pharmacological and putative physiological actions. They are overlapping, but physiologically the hypothalamic paracrine local secretion, distribution and action of the putative GHRP hormone inside the blood-brain barrier would have special implications, as would its presence, amount and timing of secretion into the portal system. Pharmacologically the blood-brain barrier also needs special consideration. This is because the hypothalamic and pituitary actions of GHRP are complementary, and low-dose GHRP administered peripherally would be outside the blood-brain barrier and thus perhaps only reach specific hypothalamic anatomical sites.

More recently, demonstration of GHRP action on anatomical sites of the hypothalamus involved in regulation of GH secretion, in particular the arcuate neurons, has been another important milestone [20–26]. Evidence obtained by Dickson et al. [23] and Kamegai et al. [25] recorded in figure 2 demonstrates conclusively a GHRP action on GHRH neurons but not SRIF neurons. However, equally notable is that GHRP stimulates only a small proportion (20%) of the GHRH arcuate neurons and that most of the GHRP-stimulated arcuate neurons are located in the non-GHRH ventral

medial part of the nucleus. These neurons are still incompletely identified in terms of type and function. So far neuropeptide-Y (NPY)-containing arcuate neurons have been identified as the most common neuron stimulated by GHRP. Also, a subpopulation of GHRP-responsive arcuate neurons are inhibited by the SRIF analogue, octreotide, indicating an aspect of the GHRP-SRIF relationship at the hypothalamic level in need of further detailed study [26]. The finding that the hypothalamic action of GHRP could be demonstrated in hypophysectomized rats eliminated the possibility that GH mediated these observed GHRP hypothalamic actions [25]. GHRP stimulation of arcuate neurons of the *lit/lit* mouse demonstrates that the GHRP action on the hypothalamus is independent of a GHRH action [23]. Because of a GHRH receptor mutation, this mutant dwarf GH-deficient mouse is nonresponsive to GHRH. A final pertinent result reflecting a hypothalamic action of GHRP is the rise of GHRH in hypophyseal portal blood after GHRP administration [27].

GHRP-GHS receptor

The Merck group's accomplishments on GHRP-GHSs under the direction of Roy Smith have been exceptional at both the basic and clinical levels [28]. In 1996, they accomplished the important milestone of cloning the GHRP-GHS receptor [29]. It is a seven-transmembrane G protein-coupled receptor expressed by a single highly conserved gene in the human, chimpanzee, pig, cow, rat and mouse. In 1998, results of Scott Feighner and his Merck colleagues [30] demonstrated that single mutations in transmembranes 2,3 and 5,6 affect binding and activation of the GHRP-GH secretagogues. They proposed a three-dimensional receptor model of the spiropiperidine and benzolactam nonpeptide GHS and the peptide GHRP-6. Mutating glutamic acid to glutamine at position 124 in transmembrane 3 resulted in a nonfunctional receptor for each of the three different chemical types of GHRPs. Since each GHRP has an essential positive charged N atom at the N terminus, the nonfunctional receptor was explained by eliminating the counter ion interaction between these three GH secretagogues and the receptor. The transmembrane 2,5 and 6 mutations induced different effects on the binding and activation of these three chemically different GHRPs. This led to the interesting speculation that these three GHRPs probably bind to the receptor site by different orientations.

By using a riboprobe of the entire length of the receptor, it appears the receptor is mainly distributed within the central nervous system (CNS). Besides the selective localization of these receptors on the somatotroph cell of the pituitary, also exciting and relevant to the CNS action of GHRP are studies on the location and regulation of the GHRP-GHS-R (GH-secretagogue receptor) within the CNS. In addition to possible other anatomical sites of the brain, the transcripts of the GHRP-GHS-R have been found to be prominently expressed in the arcuate and ventromedial nucleus (VMN) as well as the hippocampus, indicating that these are probably major sites of GHRP action [31, 32]. Most notable are the studies of Bennett and Robinson et al. (fig. 3) which reveal the following series of important findings. The receptors at the above three prominent anatomical sites appear to be regulated by GH, because at each site the receptor is increased by GH deficiency and decreased by GH excess. Also, in the arcuate nucleus, GH decreased

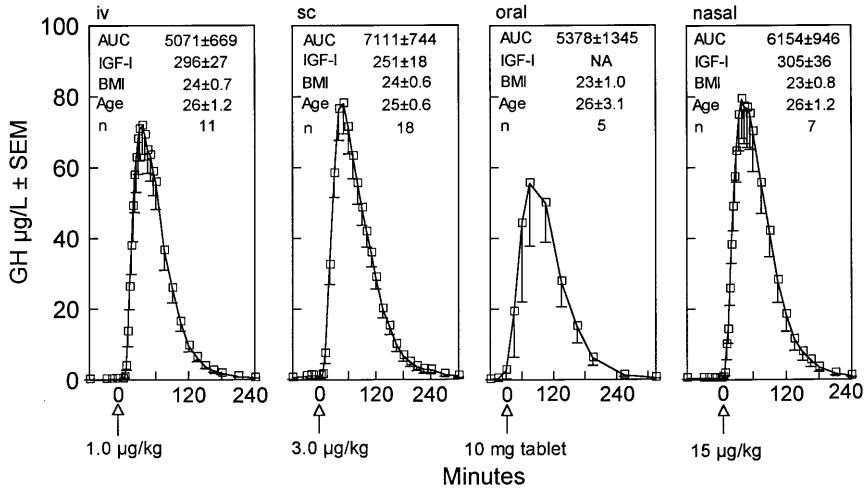

Figure 5. Time response curve of GHRP-2 after i.v., s.c., oral and nasal administration in normal young men. Values are mean ± SEM. AUC, area under the curve; IGF-I (µg/l); BMI, body mass index.

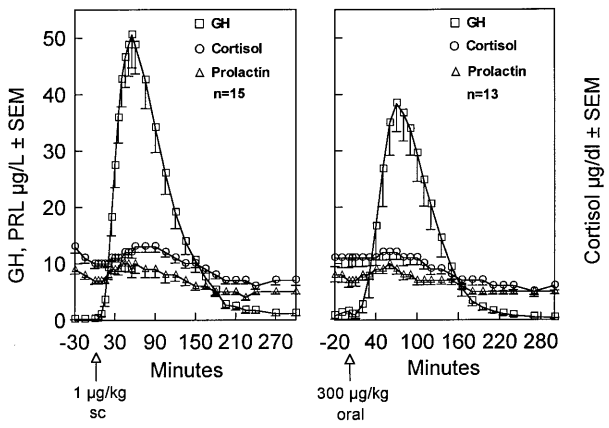

Figure 6. GH, PRL and cortisol levels in normal young men after 1 µg/kg s.c. (left panel) and 300 µg/kg oral GHRP-2 (right panel). Values are mean ± SEM.

the transcripts for GHRP-GHS-R as well as GHRH in parallel, but GH has a nonparallel effect on GHRP-GHS-R and NPY transcripts in that the former was decreased whereas the latter was increased. Continuous GHRP-6 administration did not alter expression of the GHRP-GHS-R in the arcuate or VMN. In the VMN, the GHRP-GHS-R transcripts are higher in the female than the male rat. Even though the GHRP-GHS-R is prominent in the VMN, GHRP does not stimulate c-*fos* in the VMN, which is in contrast to c-*fos* stimulation in the arcuate nucleus.

Using different techniques, other groups have demonstrated GHRPs in various peripheral tissues, including the heart. Ong et al. [33] reported evidence for a pituitary subtype GHRP-R.

Already the receptor has been identified in pathological tissue. The GHRP-GHS-R has been detected in human pituitary tumours, that is, somatotropinomas and prolactinomas but not functionless pituitary tumours, and the tumours with these receptors are responsive to the action of GHRP [34, 35]. Similarly, the rat pituitary cell line GH_3 expresses the GHRP-GHS-R [34]. More recently, messenger RNA (mRNA) for the GHRP-GHS-R has been reported to be expressed in human foetal pituitary at 18 and 31 weeks of age, and also it has been shown to be functionally responsive to GHRP in vitro [36].

Because of the current focus and recent exciting results on the hypothalamic action of GHRP, it is necessary to reemphasize that the pituitary action of GHRP needs to be included in a conceptual model of how GHRP acts to release GH [37]. It is relevant GHRP specifically releases GH by a direct pituitary action via a specific receptor and intracellular pathway in multiple animal species as well as in humans. As stated above, functional GHRP receptors have been demonstrated recently in human foetal pituitaries and human pituitary tumour cells. Also, structure-activity studies have revealed many specific chemical requirements of the GHRP-GHS on pituitary action [17]. These results strongly support the pituitary as another one of the anatomical sites of action, and it should be included in the physiological and pharmacological conceptual models on the action of the putative GHRP-like hormone and the various GHRP-GHSs [16, 18].

Clinical effects

The following series of clinical results have been selected in order to portray the scope of the effects of GHRP on GH release in humans and, by implication, are reasons for believing GHRP may be important clinically [38, 39]. In addition, they may reveal new basic knowledge about the regulation and secretion of GH. Our first GHRP (GHRP-6) studies in humans were performed in collaboration with Michael Thorner in 1988 [40]. At the same time, Ilson et al. [41] also reported clinical results of GHRP-6 in normal young men.

Results of the GH responses induced by GHRP-6, -1, -2 are shown in figure 4. All three GHRPs induced more GH release than GHRH at an intravenous (i.v.) bolus dose of 1 µg/kg. The greater GH release implies that new mechanisms are involved in the action of GHRP, an

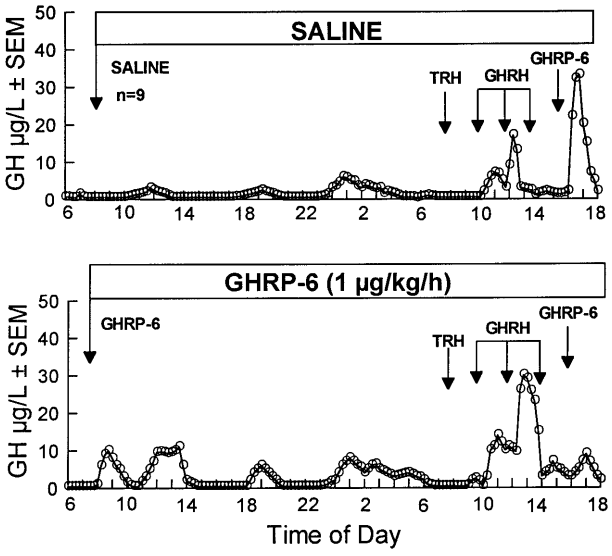

Figure 7. Effect of 36-h infusion of saline and GHRP-6 in normal young men. Acute i.v. injections of TRH (50 µg), GHRH (1 µg/kg) and GHRP-6 (1 µg/kg) were given at the times designated by the arrows [43]. Reprinted with permission from: Jaffe C. A., Ho J., Demott-Friberg R., Bowers C. Y. and Barkan A. L. (1993) Effects of a prolonged growth hormone (GH)-releasing peptide infusion on pulsatile GH secretion in normal men. J. Clin. Endocrinol. Metab. **77:** 1641–1647, © 1998 The Endocrine Society, Bethesda, MD.

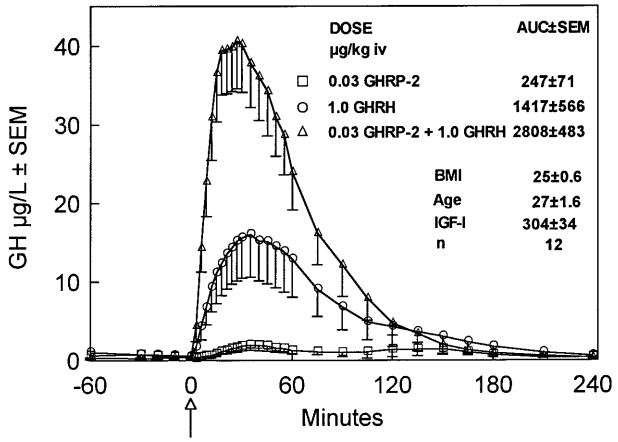

Figure 8. Effect of low subthreshold dose of GHRP-2 (0.03 µg/kg) alone and with high-dose GHRH (1 µg/kg) on the synergistic release of GH in nine normal young men. Values are the mean ± SEM. AUC, area under the curve; IGF-I (µg/l); BMI, body mass index.

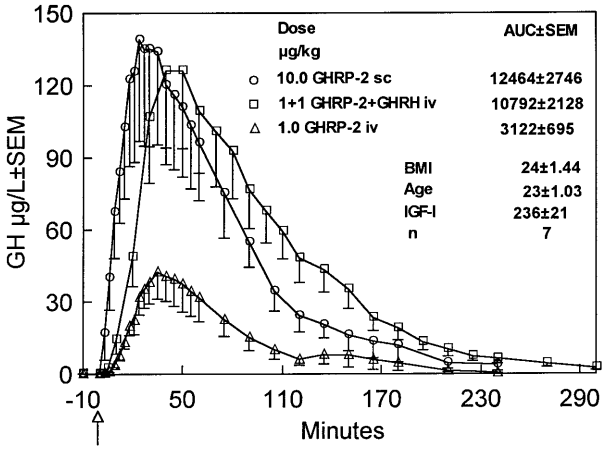

Figure 9. Effect of 10 µg/kg s.c. GHRP-2 vs. 1.0 µg/kg i.v. and 1 + 1 µg/kg GHRP-2 + GHRH i.v. in the same seven normal young men. Values are the mean ± SEM. AUC, area under the curve; IGF-I (µg/l); BMI, body mass index.

implication that has subsequently been substantiated. Recorded in figure 5 are data which support that a valuable clinical feature of GHRP is the release of GH by all routes of administration. GHRP-2 released GH in normal young men after i.v., subcutaneous (s.c.), intranasal and oral administration. In the first clinical study of GHRP-6 in normal young men, a small but definite rise of cortisol and PRL was induced [40]. As recorded in figure 6, similar results were obtained when 1 µg/kg s.c. or 300 µg/kg oral GHRP-2 was administered to normal young men.

We, as well as other investigators, have demonstrated that repeated frequent administration of GHRP markedly inhibits the GH response in rats and humans.

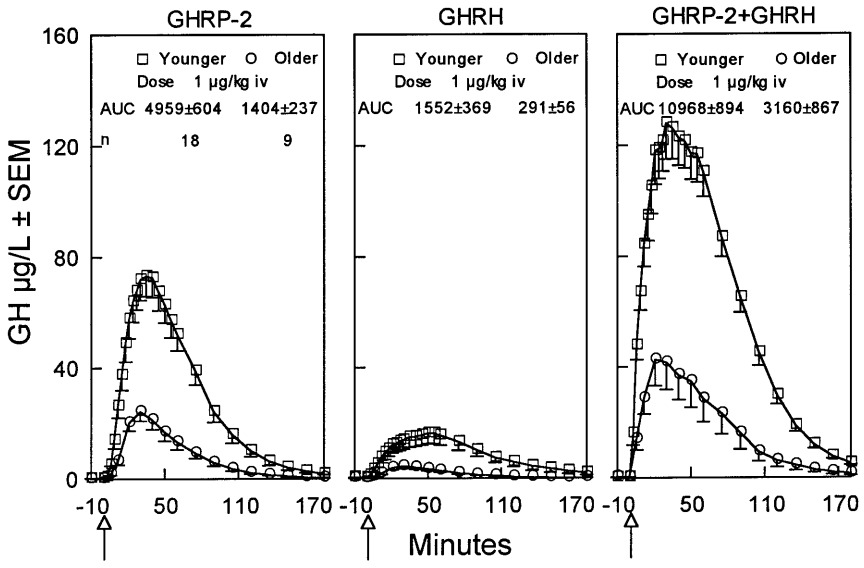

Figure 10. GH response to GHRP-2, GHRH and GHRP-2 + GHRH in normal younger and older men. Values are the mean ± SEM. AUC, area under the curve; IGF-I, 303 ± 32 µg/l (younger) and 159 ± 15 (older); BMI (body mass index) = 25 ± 0.6 (younger) and 27 ± 0.6 (older). Age = 25 ± 1.0 (younger) and 66 ± 2.0 (older) [18]. Reprinted with permission from: Bowers C. Y. (1998) GHRP + GHRH synergistic release of GH: scope and implication. In: Growth Hormone Secretagogues, pp. 1–25, Bercu B. and Walker R. (eds),

As demonstrated in figure 7, the results of continuous GHRP-6 administration to normal young men are particularly noteworthy [42, 43]. A priori, it was projected that the GH response to continuous GHRP administration in humans, as observed in rats, would be desensitized. As predicted, in the study of Jaffe et al., the GH response to i.v. bolus GHRP-6 in normal young men was markedly desensitized at the end of the continuous infusion for 36 h, but the increase in the normal spontaneous pulsatile secretion of GH during the entire GHRP infusion period was not predicted. Despite the desensitization of the GHRP-GH response, as indicated by the decreased GH response to i.v. bolus GHRP at the end of the GHRP infusion period, the amplitude but not the frequency of the spontaneous GH pulses continues to be increased, underscoring a unique physiological type of action of GHRP on GH secretion. Another noteworthy finding in this study was augmentation of the GH response to i.v. bolus GHRH at the end of the GHRP infusion period, at least during the first two of the three repeated i.v. bolus injections of GHRH. These results demonstrate that desensitization and sensitization of the GHRP action can occur concomitantly. However, desensitization of the GH response to GHRP is partial, whereas at the same time the GH response to GHRH continues to be sensitized or augmented.

In principle, these results might have been predicted from the study of Clark and Robinson reported in 1989 in which conscious rats were continuously infused with GHRP-6 for 8 h and i.v. bolus GHRH administered each hour [44]. Each GHRH bolus injection elicited GH release during the GHRP infusion, but not without it, indicating that GHRP sensitized the pituitary action of GHRH on GH release. The actual mechanism involved is still unclear, but it is now known that continuous GHRP infusion and longer-acting GHRP-GHSs, that is, the Merck peptidomimetic MK-0677, have been found to enhance GH pulsatile secretion in normal subjects. The many important GHRP-GHS results of the Merck group have been reviewed by Smith et al. as well as Patchett et al. [28, 45, 46]. These studies again support that the longer-acting effect of the GHRP-GHS does not completely desensitize the GH response.

Particularly noteworthy are the comparative effects of the GH-releasing action of low- vs. high-dose GHRP, because they reveal novel aspects of the GHRP action [18, 39, 47]. Figure 8 shows the GH response to a very low dose of GHRP-2, 0.03 μg/kg (~2 μg/subject), alone and together with a 1 μg/kg maximal dose of GHRH in normal young men. Even at a subthreshold GH-releasing dosage, GHRP-2 augmented the GHRH-GH response. Conclusions from these results are as follows. The lack of a GH response to 2 μg of GHRP supports that the action of the subthreshold GH-releasing dose of GHRP is on the hypothalamus rather than the pituitary. Because of the low dose of GHRP-2 and the blood-brain barrier, the anatomical site of action of the low dose is postulated to be on the hypothalamic median eminence. In addition, it is hypothesized that the

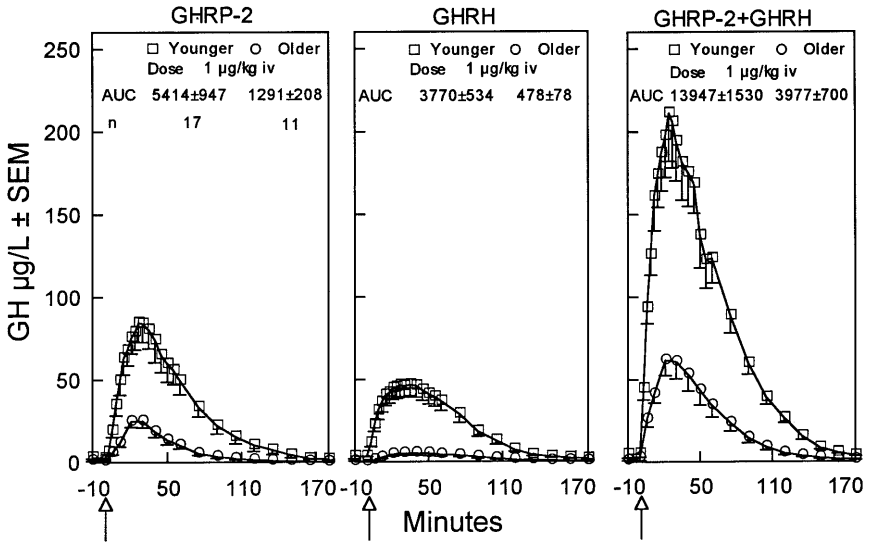

Figure 11. GH response to GHRP-2, GHRH and GHRP-2 + GHRH in normal younger and older women. Values are the mean ± SEM. AUC, area under the curve; IGF-I = 265 ± 22 μg/l (younger) and 114 ± 11 (older); BMI (body mass index) = 25 ± 0.5 (younger) and 26 ± 0.7 (older). Age = 25 ± 1.1 (younger) and 67 ± 2.0 (older) [18]. Reprinted with permission from: Bowers C. Y. (1998) GHRP + GHRH synergistic release of GH: scope and implication. In: Growth Hormone Secretagogues, pp. 1–25, Bercu B. and Walker R. (eds), © 1998 Marcel Dekker Inc., New York.

Table 3. On the pathophysiology of older men and women with decreased GH secretion.

Peptide i.v. bolus	Dose μg/kg	Mild peak GH ng/ml	Moderate peak GH ng/ml	Severe peak GH ng/ml
GHRH	1.0	14.7 ± 2.7	4.2 ± 0.2	3.3 ± 1.2
GHRP-2	0.1	6.3 ± 2.5	3.5 ± 1.2	1.2 ± 0.4
GHRP-2 + GHRH	0.1 + 1.0	38.6 ± 6.5	24.0 ± 4.2	7.1 ± 0.9
GHRP-2	1.0	41.2 ± 4.7	32.8 ± 5.1	14.3 ± 2.3
			AUC GH μg/l · 4 h	
GHRH	1.0	1011 ± 179	352 ± 46	263 ± 67
GHRP-2	0.1	376 ± 95	217 ± 54	103 ± 34
GHRP-2 + GHRH	0.1 + 1.0	2196 ± 402	1402 ± 272	391 ± 27
GHRP-2	1.0	2426 ± 344	1890 ± 327	706 ± 117
IGF-I (μg/l)		151.0 ± 17.0	121.0 ± 11.0	117.0 ± 18.0
Age (years)		63.8 ± 2.3	67.8 ± 1.3	67.3 ± 3.1
BMI		25.3 ± 0.7	26.7 ± 0.2	26.5 ± 1.8
n		5	11	4

Values: mean ± SEM. BMI, body mass index; AUC, area under the curve.

hypothalamic GHRP action is not due to release of endogenous GHRH, because the excess exogenous GHRH administration would obviate any effect of endogenous GHRH that may be released by GHRP. Other previous relevant findings are that the GHRP-SRIF in vitro and in vivo GH-releasing action in animals and humans does not support that low-dose GHRP involves an action on SRIF release from the hypothalmaus or inhibition of the action of SRIF on the pituitary to release GH. To explain these results in humans, we have hypothesized that low-dose GHRP releases U-factor (unknown factor) from the hypothalamus, and that by a pituitary action U-factor augments the GH-releasing action of GHRH. Also envisioned is that U-factor may be involved in the action of the putative GHRP-like hormone in the physiological regulation of pulsatile secretion of GH.

Results in figure 9 reveal that a novel effect on GH release also occurs when a large pharmacological dose of GHRP is administered. In this study a high dose of 10 μg/kg s.c. released an inordinate amount of GH in normal young men. In these same men the GH response to i.v. bolus 1 μg/kg GHRP-2 as well as 1 μg/kg GHRP-2 + 1 μg/kg GHRH was determined. Since the GH response to 1 μg/kg GHRH alone is always much lower than the GH response to 10 μg/kg GHRP-2 in normal young men, it is not possible to explain the later response by a hypothalamic action of GHRP on the release of endogenous GHRH alone. Our interpretation of these results is that endogenous GHRH as well as U-factor is released via the hypothalamic action of high-dose GHRP and that GHRP, GHRH and U-factor act together on the pituitary to release GH synergistically. In these same men, GHRP-2 + GHRH at 1 μg/kg releases the same inordinately large amount of GH as 10 μg/kg GHRP-2. These results support the hypothesis that a high dose of 10 μg/kg GHRP releases endogenous GHRH. In part, the synergistic response probably involves attenuation of the inhibitory pituitary action of SRIF on GH release by the pituitary action of the three peptides. In studies in humans by Massoud et al. [48] combined GHRP + GHRH was more effective

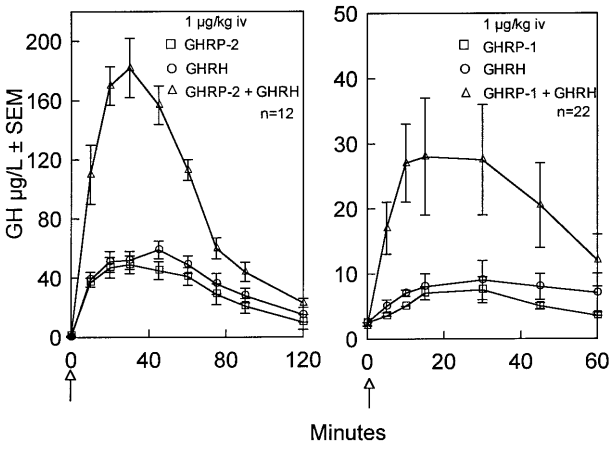

Figure 12. Synergism in short-statured children. Acute test to GHRP-2, GHRH and GHRP-2 + GHRH (left panel) [55] and GHRP-1, GHRH and GHRP-1 + GHRH (right panel) [53]. Reprinted with permission from: (left panel) Pihoker C., Middleton R., Reynolds G. A., Bowers C. Y. and Badger T. M. (1995) Diagnostic studies with intravenous and intranasal growth hormone releasing peptide-2 in children of short stature. J. Clin. Endocrinol. Metab. **80:** 2987–2992, © 1998 The Endocrine Society, Bethesda, MD, and (right panel) Mericq V., Cassorla F., Garcia H., Avila A., Bowers C. Y. and Merriam G. (1995) Growth hormone responses to growth hormone releasing peptide (GHRP) and to growth hormone releasing hormone (GHRH) in growth hormone deficient children (GHD). J. Clin. Endocrinol. Metab. **80:** 1681–1684, © 1998 The Endocrine Society, Bethesda, MD.

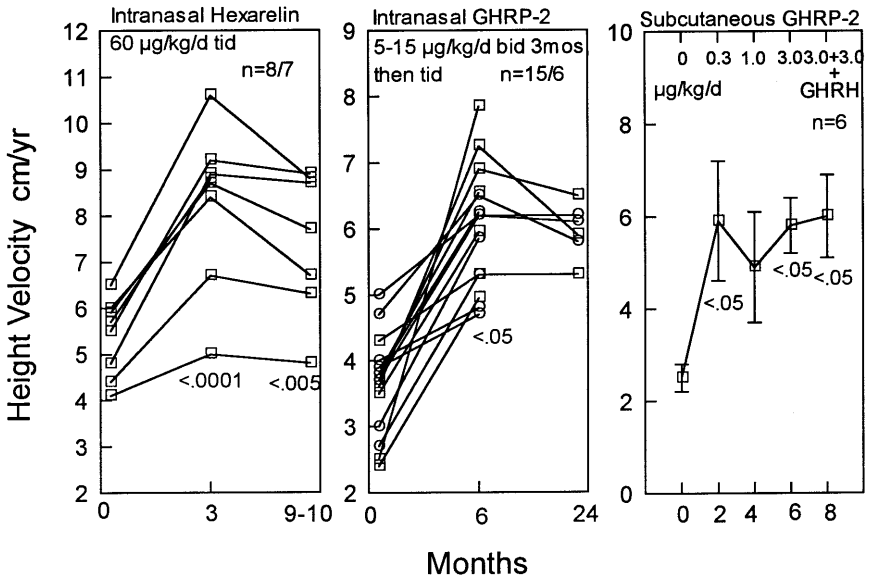

Figure 13. Effect of GHRPs on height velocity in short-statured children. Height velocity ranged from 2.1 to 3.2 cm/year [49–51]. Reprinted with permission from: (left panel) Klinger B., Silbergeld A., Deghenghi R., Frenkel J. and Laron Z. (1996) Desensitization from long-term intranasal treatment with hexarelin does not interfere with the biological effects of this growth hormone releasing peptide in short chidren. Eur. J. Endocrinol. **134:** 716–719, © 1998 Society of the European Journal of Endocrinology. (Middle panel) Pihoker C., Badger T. M., Reynolds G. A. and Bowers C. Y. (1997) Treatment effects of intranasal growth hormone releasing peptide-2 in children with short stature. J. Endocrinol. **155:** 79–86, © 1998 The Endocrine Society, Bethesda, MD. (Right panel) Mericq V., Salazar T., Avila A., Inguez G., Bowers C. Y., Cassorla F. et al. (1998) Treatment with growth hormone releasing peptide accelerates growth of growth hormone-deficient children. J. Clin. Endocrinol. Metab. **83:** 2355–2360, © 1998 The Endocrine Society, Bethesda, MD.

in attenuating the effect of SRIF on GH release than the peptides alone.

Results of the synergistic release of GH induced by i.v. bolus 1 µg/kg GHRP-2 + GHRH in normal younger and older men and women are recorded in figures 10 and 11, respectively. A synergistic GH response to the two peptides together occurred in all subjects. In addition, the GH responses to GHRP-2, GHRH and GHRP-2 + GHRH were notably less in the older than in the younger subjects.

The following are possible pathophysiological mechanisms responsible for decreased GH secretion in normal older subjects. In the past, the hypothesis has been that there is a decrease of GHRH secretion or an increase of SRIF secretion to account for decreased GH secretion. We now propose that decreased secretion of the putative GHRP-like hormone may be one reason GH secretion is decreased in some older subjects. Also, it is possible that this may be due to a mixture of the above or none of the above.

To investigate the pathophysiology of the decreased secretion of GH in normal elderly subjects and the possibility of a putative GHRP-like hormone deficiency, GH responses to i.v. bolus 1 µg/kg of GHRH or GHRP-2, 0.1 µg/kg of GHRP-2 and 0.1 µg/kg of GHRP-2 + 1 µg/kg of GHRH were determined. In this study, the GH results of 20 normal older subjects were categorized into three groups according to the degree of the decreased peak GH response to GHRP-2 + GHRH, that is, mild (5 subjects), moderate (11 subjects), marked (4 subjects). As recorded in table 3 the peak GH response of the combined peptides in the mildly impaired group was 39 µg/l, whereas this value was only 2 µg/l in the markedly impaired group and 24 µg/l in the moderately impaired GH response group. In regard to new insight into the pathophysiology of decreased secretion of GH in older subjects, the most meaningful results were obtained from the moderately impaired GH response group. This insight was derived from the results of a very low peak GH response of 4 µg/l to the 1 µg/kg dose of GHRH as well as the reversal of this decreased GH response by administrating the low dose of GHRP-2 in combination with 1 µg/kg of GHRH. The impaired pituitary response to 1 µg/kg of GHRH implies a primary pituitary disorder, perhaps due to decreased pituitary stores of GH secondary to decreased endogenous GHRH secretion or possibly due to excess SRIF secretion; however, the dramatic reversal by 0.1 µg/kg of GHRP-2 administered together with 1 µg/kg of GHRH weighs strongly against either one of these possibilities. Low-dose GHRP is envisioned to act on the hypothalamus to release U-factor and together

with GHRH reverse the impaired pituitary GH response to GHRH. The results clearly demonstrate that decreased pituitary GH stores are not the reason GHRH is ineffective. Also, it is apparent that even if low-dose GHRP-2 released GHRH by a hypothalamic action, the impaired pituitary response to exogenous GHRH reveals that any endogenous GHRH released would be ineffective. Additionally, from data of our

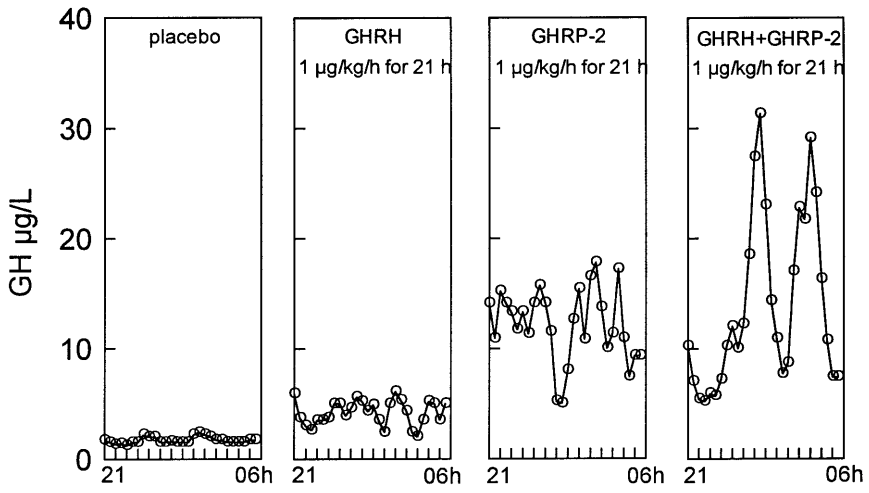

Figure 14. Continuous infusion of GHRP-2 in critically ill patients, $n = 20$ [57, 58]. Reprinted with permission from: Van den Berghe G., de Zegher F., Veldhuis J. D., Wouters P., Awouters M., Verbruggen W. et al. (1997) The somatotropic axis in critical illness: effect of continuous GHRH and GHRP-2 infusion. J. Clin. Endocrinol. Metab. **82:** 590–599 and Van den Berghe G., De Zegher F., Baxter R. C., Veldhuis J. D., Wouters P., Schetz M. et al. (1998) Neuroendocrinology of prolonged critical illness: effects of exogenous thyrotropin-releasing hormone and its combination with growth hormone-secretagogues. J. Clin. Endocrinol. Metab. **83:** 309–319, © 1998 The Endocrine Society, Bethesda, MD.

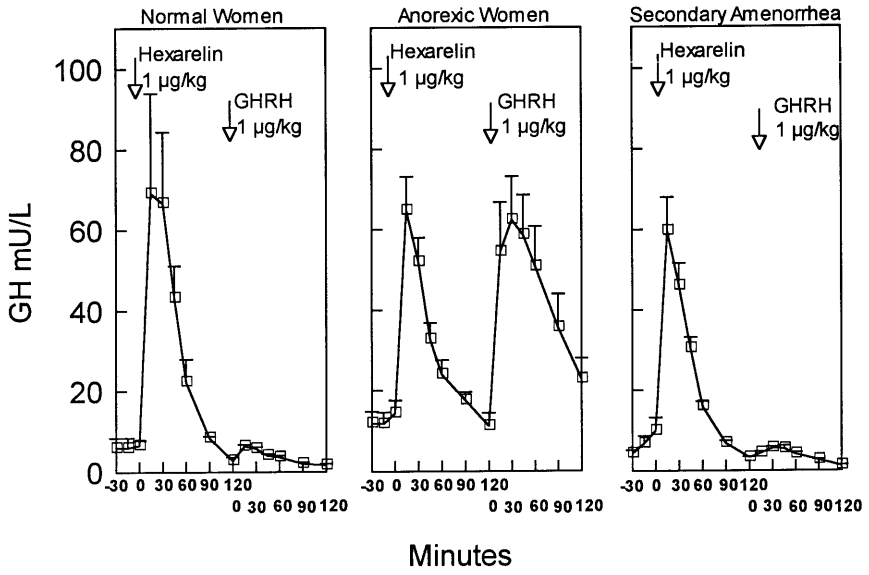

Figure 15. Sequential i.v. hexarelin-GHRH administration in normal women ($n = 6$); age $= 24 \pm 2$, BMI $= 22 \pm 1$ (left panel); anorexic women ($n = 14$), age $= 20 \pm 1.4$, BMI $= 15 \pm 0.4$ (middle panel); and women with secondary amenorrhoea ($n = 7$), age $= 21 \pm 1$, BMI $= 17 \pm 0.6$ (right panel) [60]. Reprinted with permission from: Popovic V., Micic D., Djurovic M., Obradovic S., Casanueva F. F. and Dieguez C. (1997) Absence of desensitization by hexarelin to subsequent GH releasing hormone-mediated GH secretion in patients with anorexia nervosa. Clin. Endocrinol. **46:** 539–543, © 1998 The Endocrine Society, Bethesda, MD.

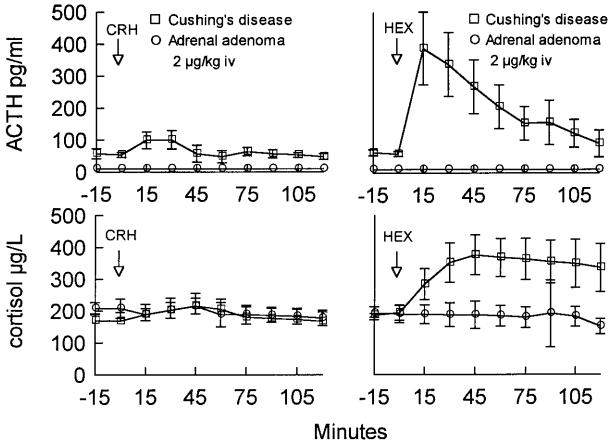

Figure 16. Effect of hexarelin and corticotropin releasing factor (CRF) in patients with Cushing's disease ($n = 10$) and adrenal adenomas ($n = 5$) on ACTH and cortisol [60]. Reprinted with permission from: Popovic V., Micic D., Djurovic M., Obradovic S., Casanueva F. F. and Dieguez C. (1997) Absence of desensitization by hexarelin to subsequent GH releasing hormone-mediated GH secretion in patients with anorexia nervosa. Clin. Endocrinol. **46:** 539–543, © 1998 The Endocrine Society, Bethesda, MD.

previous GHRP-2-SRIF in vitro and in vivo studies, a low dose of GHRP would not be expected to attenuate the pituitary inhibitory action of SRIF on GH release. Another meaningful result is the relatively large amount of GH release induced by 1 µg/kg of GHRP-2 alone. Since in vivo GHRP requires endogenous GHRH secretion in order to induce GH release, this particular result indicates endogenous GHRH is being secreted but the pituitary GHRH response of these older subjects is impaired.

Collectively, these results indicate that in some normal older subjects GH secretion is decreased not because of decreased secretion of endogenous GHRH or an excess secretion of SRIF, but rather it is postulated that this may be due to a possible deficiency of the putative GHRP-like hypothalamic hormone. Whether a deficiency of the putative GHRP-like hormone might play a role in the mild and severely impaired GH response groups is more difficult to evaluate because of the smaller number of observations and the more borderline decreased values of the mild group. Studies to prove or disprove this hypothesis are ongoing in our clinical research centre.

Already three studies have demonstrated that chronic administration of GHRP for 6 to 24 months to short-statured children with various degrees of GH deficiency augments height velocity [49–51], and four studies have been performed on the possible acute diagnostic use of GHRP in short-statured children [52–55]. Results of Pihoker et al. [55] and Mericq et al. [53] on diagnostic studies are recorded in figure 12. As recorded in figure 13, GHRP studies of Laron et al. [49], Pihoker et al. [50] and Mericq et al. [51] demonstrate the effects of chronic GHRPs after intranasal or subcutaneous administration. The GHRP effects on height velocity (2.1–3.2 cm/year) are less than those induced with recombinent human GH, but the GHRP approach is yet to be optimized in terms of the particular GHRP formulation, dosage, time and frequency of administration. Furthermore, since the mechanism of action of GHRP has increasingly become better understood, more rational clinical approaches will be proposed in the future. Current studies in children and elderly subjects include chronic administration of GHRP-2 as well as the Merck GHRP-GHS (MK-0677).

In a series of studies Van den Berghe et al. [56–58] demonstrated that nightly GH secretion during prolonged critical illness is characterized by a high number of small secretory bursts superimposed on low basal secretion in the presence of low-serum IGF-I levels. Both basal and pulsatile GH secretion, as recorded in figure 14 was increased moderately by continuous infusion of GHRH, substantially by GHRP-2 and strikingly by GHRP-2 + GHRH [57, 58]. GHRP-2 alone or together with GHRH robustly raised serum IGF-I levels within 24 h. It has been concluded that these observations open perspectives for the GHRP-GHSs as potential antagonists of the catabolic state in critical care patients.

Other interesting studies have been performed by Cordido et al. in obese subjects [59] and in women with anorexia nervosa and secondary amenorrhoea by Popovic et al. [60]. In the latter study, as shown in figure 15, hexarelin and GHRH were administered sequentially 2 h apart. In normal women and women with secondary amenorrhoea, hexarelin inhibited subsequent GH responses to GHRH. Since hexarelin did not inhibit GHRH-GH responses in women with anorexia nervosa, it was suggested by these investigators that this may be a new way to differentiate young women with anorexia nervosa from those with secondary amenorrhoea.

Ghigo et al. have performed a considerable number of unique GHRP studies with hexarelin [61]. As recorded in the right panel of figure 16, hexarelin increased serum ACTH and cortisol levels in patients with Cushing's disease, but not patients with Cushing's syndrome due to an adrenal adenoma [62]. As recorded in the left panel, CRH had little or no effect in both types of patients.

Summary

Proof of principle of the GHRP clinical approach appears to be established, and now optimization of this

approach is needed. Of particular importance, especially in normal older men and women with decreased GH secretion, is that GHRP induces physiological secretion of GH. Because the normal negative feedback GH systems are still operative in these subjects, overtreatment with GHRP would be minimized.

One reason for the sustained interest in GHRP by many investigators is that it has both a theoretical and a practical aspect. From the theoretical standpoint, results are continuing to accumulate on the possible presence and role of the putative GHRP-like hormone, which may be involved in the physiological regulation of GH secretion. From the practical standpoint, the interest in GHRP has evolved mainly from effects in humans. GHRP releases GH in humans regardless of age or sex, and sometimes the effects on GH release have been novel and imply that it may be of both diagnostic and therapeutic value.

Acknowledgements. This work was supported in part by National Institutes of Health grants AM-06164, DK40202 and PHS RR05096-09 (GCRC). Special appreciation is expressed to Dr. Granda-Ayala, to the technicians and the fellows of the endocrinology and metabolism section of the department of medicine, and to Robin Alexander for typing the manuscript. Special thanks are expressed to Dr. Fred Wagner at BioNebraska for supplying GHRH 1-44NH_2.

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