REVIEW

Growth hormone-releasing peptides

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Abstract

Growth hormone-releasing peptides (GHRPs) are synthetic, non-natural peptides endowed with potent stimulatory effects on somatotrope secretion in animals and humans. They have no structural homology with GHRH and act via specific receptors present either at the pituitary or the hypothalamic level both in animals and in humans. The GHRP receptor has recently been cloned and, interestingly, it does not show sequence homology with other G-protein-coupled receptors known so far. This evidence strongly suggests the existence of a natural GHRP-like ligand which, however, has not yet been found. The mechanisms underlying the GHRP effect are still unclear. At present, several data favor the hypothesis that GHRPs could act by counteracting somatostatinergic activity both at the pituitary and the hypothalamic level and/or, at least partially, via a GHRH-mediated mechanism. However, the possibility that GHRPs act via an unknown hypothalamic factor (U factor) is still open. GHRP-6 was the first hexapeptide to be extensively studied in humans. More recently, a heptapeptide, GHRP-1, and two other hexapeptides, GHRP-2 and Hexarelin, have been synthesized and are now available for human studies. Moreover, non-peptidyl GHRP mimetics have been developed which act via GHRP receptors and their effects have been clearly demonstrated in animals and in humans in vivo. Among non-peptidyl GHRPs, MK-0677 seems the most interesting molecule. The GH-releasing activity of GHRPs is marked and dose-related after intravenous, subcutaneous, intranasal and even oral administration. The effect of GHRPs is reproducible and undergoes partial desensitization, more during continuous infusion, less during intermittent administration; in fact, prolonged administration of GHRPs increases IGF-I levels both in animals and in humans. The GH-releasing effect of GHRPs does not depend on sex but undergoes age-related variations. It increases from birth to puberty, persists at a similar level in adulthood and decreases thereafter. By the sixth decade of life, the activity of GHRPs is reduced but it is still marked and higher than that of GHRH. The GH-releasing activity of GHRPs is synergistic with that of GHRH, is not affected by opioid receptor antagonists, such as naloxone, and is only blunted by inhibitory influences, including neurotransmitters, glucose, free fatty acids, glucocorticoids, recombinant human GH and even exogenous somatostatin, which are known to almost abolish the effect of GHRH. GHRPs maintain their GH-releasing effect in somatotrope hypersecretory states such as in acromegaly, anorexia nervosa and hyperthyroidism. On the other hand, their good GH-releasing activity has been shown in some but not in other somatotrope hypersecretory states. In fact, reduced GH responses after GHRP administration have been reported in idiopathic GH deficiency as well as in idiopathic short stature, in obesity and in hypothyroidism, while in patients with pituitary stalk disconnection or Cushing’s syndrome the somatotrope responsiveness to GHRPs is almost absent. In short children an increase in height velocity has also been reported during chronic GHRP treatment. Thus, based on their marked GH-releasing effect even after oral administration, GHRPs offer their own clinical usefulness for treatment of some GH hyposecretory states.

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Introduction

The first peptides of the growth hormone-releasing peptide (GHRP) family were invented rather than isolated in 1977 (1). Although derivatives of the pentapeptide met-enkephalin, they were devoid of opiate activity, but showed low potency on the pituitary in vitro and were inactive in vivo (2, 3). Guided by theoretical low energy conformational calculations, synthesis of new peptides resulted in the potent hexapeptide GHRP-6 (His-D-Trp-Ala-Trp-D-Phe-Lys-NH2) which releases GH in vitro and in vivo (4–9). The dose-dependent GH-releasing activity of GHRP-6 has been demonstrated in several species (4, 9–12) and
in man after intravenous (i.v.), subcutaneous (s.c.), intranasal (i.n.) and even oral (p.o.) administration (13–19).

The potent GH-releasing effect seen even after oral administration prompted the research in this field. Now the GHRP family includes more potent analogs to GHRP-6, such as a heptapeptide, GHRP-1 (Ala-His-D-beta-Nal-Ala-Trp-D-Phe-Lys-NH₂), and two hexapeptides, GHRP-2 (D-Ala-D-beta-Nal-Ala-Trp-D-Phe-Lys-NH₂) and Hexarelin (His-D-2-methyl-Trp-Ala-Trp-D-Phe-Lys-NH₂) whose activities have been extensively studied in animals and humans (18, 20–26). As all these peptides have the disadvantage that they are less than 1% orally bioavailable, other GHRP mimetics, having structures more amenable to chemical modifications and optimization of oral bioavailability, have been synthesized. Among them, non-peptidyl GHRPs (L-692,429, L-692,585, L-700,653, L-163,191 or MK-0677) have been studied in animals (27–36) and even in man (33, 37–41). More recently, some cyclic peptides as well as peptido-, tetra-, and pseudotriptides have also been synthesized and studied in animals (42–44).

**GHRP receptors and signal transduction pathway identification**

A wealth of data indicates that the actions of GHRPs and non-peptidyl GHRP mimetics are mediated by specific receptors and intracellular mechanisms distinct from those of growth hormone-releasing hormone (GHRH). In fact, the human GHRP receptor has recently been cloned. It is encoded by a rare mRNA with a predicted open reading frame of 366 amino acids with a transmembrane topography typified by the G-protein-coupled receptor family. Notably, the receptor sequence does not show significant homology with other receptors known so far, while receptor transcripts are expressed in the pituitary and the hypothalamus (45).

A specific high-affinity binding site that mediates the activity of GHRPs and non-peptidyl GHRP mimetics has been identified in anterior pituitary and hypothalamic membranes of rat and pig (46–49). The binding affinity of these structurally different GH secretagogues to the same receptor is correlated with GH stimulatory effect (49). The binding is Mg⁺⁺-dependent, is inhibited by stable GTP analogs and is not displaced by GHRH, somatostatin, met-enkephalin, substance P, galanin, gonadotropin-releasing hormone, thyrotropin, PACAP-38, gastrin-releasing peptide, melanocyte-stimulating hormone and neurotransmitters (46–49).

We have recently extended the study on the presence of GHRP receptors in man (50, 51) in whom these compounds have been shown to have their highest GH-releasing effect (18, 20, 24, 26). Among various tissues tested, the hypothalamus and the pituitary gland of adult subjects showed the highest specific GHRP binding. Well detectable specific GHRP binding was also found in choroid plexus and cerebral cortex but not in the cerebellum or callous body (Fig. 1). No significant sex-related difference in GHRP binding to various tissues was found.

The presence of specific GHRP binding sites in human hypothalamus and pituitary gland confirms that these tissues are primary targets of GHRP actions, in agreement with studies both in animals (6, 7, 52–60) and in humans (14, 19, 61–63) addressing hormonal activities of GHRPs. On the other hand, the presence of specific GHRP binding sites in human choroid plexus and cerebral cortex suggests that these peptides could cross the blood–brain barrier and influence

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**Figure 1** Specific binding of 125I-Tyr-Ala-Hexarelin to membranes from the pituitary gland and different regions of the brain of adult male subjects. Specific binding was calculated as the difference between binding in the absence and in the presence of excess unlabeled Tyr-Ala-Hexarelin. Values represent means ± s.d. of four subjects.
some 'non-neuroendocrine' central nervous system (CNS) functions. In fact, it has been shown in rodents that parenteral and intracerebroventricular GHRP administration stimulate c-Fos mRNA expression in the arcuate nucleus and feeding behaviour respectively (59, 64-66). Moreover, in humans, parenteral GHRP injection has been reported to modulate sleep pattern (39, 67).

The GHRP receptors that we have detected in human brain and pituitary gland have similar basic binding properties to those described in rat and pig hypothalamus and pituitary gland (46-48). In fact, we have found that different GHRP analogs, i.e. Tyr-Ala-Hexarelin, Hexarelin and GHRP-6, are the most effective competitors of radiolabeled $^{125}$I-Tyr-Ala-Hexarelin. In contrast, the GHRP binding is not displaced by GHRH, somatostatin or galanin (50, 51).

Altogether, data showing the existence of specific GHRP binding sites at the pituitary level and within the CNS strengthen the hypothesis that GHRPs mimic an unidentified endogenous ligand which could be involved in neuroendocrine and extra-neuroendocrine activities.

In agreement with evidence that GHRPs and GHRH possess specific receptors, these peptides display different signal transduction pathways (7, 53, 54, 68, 69). In fact, whereas GHRH activates protein kinase A via stimulatory effects on adenylcyclase and cAMP production (53), GHRPs and non-peptidyl GHRP mimetics, a notable exception being GHRP-2 (70), act via intracellular mechanisms which do not include cAMP synthesis (53, 68, 69, 71). GHRPs have been reported to increase intracellular Ca$^{2+}$ and to activate ionic conductance involved in producing cell membrane depolarization (7, 53, 54, 68, 69, 71, 72). This effect is blocked by chelation of extracellular calcium or by the use of calcium channel blockers (7, 69, 71, 72). Noteworthy, GHRP-induced activation of G-protein pathways coupled to potassium channels and phospholipase C has been reported in pituitary cell cultures (49).

**Effects of GHRPs in animals: in vitro and in vivo studies**

At the pituitary level, GHRPs stimulate GH release from somatotrope cells in vitro (6, 7, 27, 28, 52-55, 68-74), probably by augmenting the number of GH-secreting cells rather than by increasing the amount of GH secreted per cell (54). Moreover, GHRPs release GH from both sparsely granulated and heavily granulated rat pituitary cell types in vitro (74). Interestingly, GHRPs have even been reported to induce GH synthesis (73).

The GH-releasing activity of GHRPs in vitro is lower than that of GHRH (6, 7, 52, 55), while their interaction has been found additive or truly synergistic (6, 52, 53, 68, 70, 71). The stimulatory effect of GHRPs on GH secretion from somatotrope cells is abolished by specific GHRP antagonists but not by GHRH antagonists (52-54), an exception being GHRP-2 (70). Although somatostatin is able to inhibit the stimulatory effect of GHRPs on GH secretion from the pituitary in vitro (4, 6, 55, 69), there is evidence suggesting that GHRPs could antagonize the inhibitory activity of somatostatin on somatotrope release by counteracting its hyperpolarizing effect on somatotrope cell membrane (54).

An important finding was that the GH-releasing activity of GHRPs was clearly higher from a hypothalo–pituitary preparation than from the pituitary (52). Thus, it became clear that the stimulatory effect of GHRPs on GH secretion is greater in vivo than in vitro (52, 57).

In vivo, GHRPs have clear synergistic effects with GHRH (9, 52), they prevent the normal cyclic refractoriness to GHRH (57) and their activity is age-dependent, being decreased in aged animals (75–78). These data, together with evidence that the GH response to direct intracerebroventricular GHRP injection is higher than that observed after systemic administration of the same dose (79), point to an important hypothalamic action of GHRPs. In agreement with this assumption, the GH-releasing effect of GHRPs and their synergism with GHRH are strongly reduced, although not abolished, in animals bearing lesions of the pituitary stalk (56, 80, 81). Noteworthy, hypothalamic ablation initially increases and subsequently reduces GHRP activity (56). Thus, normal hypothalmo–pituitary function is needed for full GHRP activity, as indicated also by clear evidence in man (see below).

At the hypothalamic level, GHRPs do not seem to have a negative influence on somatostatin, as indicated by the fact that the release of somatostatin from hypothalamic preparations is not reduced by GHRPs (82, 83). Passive immunization against somatostatin does not reduce the GH-releasing effect of GHRPs (52, 60, 73, 84), while it abolishes that of cholinergic agonists (85). Moreover, GHRPs and substances known to inhibit hypothalamic somatostatin release have a synergistic effect on GH secretion (9, 86).

The possibility that hypothalamic GHRP activity is mediated by modulation of GHRH-secreting neurons has been extensively studied. The evidence showing that GHRPs and GHRH have synergistic effects on GH secretion in vitro and in vivo (9, 28, 52, 53) does not rule out the possibility that GHRH activity mediates, at least partially, the GH-releasing effect of GHRPs. Although there are studies against a GHRH-mediated action (73), many data indicate that the integrity of GHRH activity is needed for the full GH-releasing effect of GHRPs. In fact, passive immunization against GHRH as well as pretreatment with GHRH antagonist strongly reduce the activity of GHRPs (52, 57, 60, 84, 87). GHRPs have no GH-releasing activity in the lit/lit mouse which has no functional GHRH receptors (88): interestingly, GHRPs are active in the dwarf mice where the precise mutation is not known (24). Moreover, both in GH-deficient rats and in lit/lit mice, systemically administered GHRPs activate a subpopulation of
hypothalamic arcuate neurones (59, 65). In fact, increased electrical activity and increased c-Fos-like immunoreactivity and c-Fos mRNA expression have been found at this level after GHRP administration (59, 65). Noteworthily, it is well known that the highest density of GHRH-secreting neurones is present in the arcuate nucleus (85). In agreement with a GHRH-mediated action for GHRPs, increased release of GHRH in hypophysial portal blood after GHRP administration in sheep has also been shown (58).

More recently, it has been hypothesized that the positive influence of GHRPs on GHRH-secreting neurones could be, in turn, due to functional antagonization of the inhibitory influence of somatostatin at the hypothalamic level. In fact, in rats, the GH-stimulatory effect of i.c.v. administration of GHRPs, but not that of i.v. GHRH, is blocked by i.c.v. pretreatment with octreotide (79). Thus, these data suggest that both at the hypothalamic as well as at the pituitary level (34) GHRPs could act as functional somatostatin antagonists. Noteworthily, it has recently been reported that the infusion of a GHRP antagonist inhibits not only the GH response to acute GHRH administration but even the spontaneous GH pulsatility in swine in vivo (89). If confirmed, this evidence would strongly strengthen the argument for the existence of a natural GHRP-like ligand which plays a major role in the physiological control of GH secretion.

The interaction of GHRPs with neurotransmitters and neuromodulators has also been studied. In rats, the GH-releasing effect of GHRPs is not modified by naloxone but is synergistical with d- and "LM"-enkephalin and met-enkephalin analogs (52), indicating that the activity of GHRPs is not mediated by opioid receptors. On the other hand, nitric oxide seems to play a permissive role in the GH-releasing effect of GHRPs as well as of GHRH (90). Also galanin and substance P could mediate the activity of GHRPs while bombesin could play an inhibitory role in rats (56, 91, 92).

The GH response to GHRPs is increased by pyridostigmine, a cholinesterase inhibitor, in dogs (86) as well as by propranolol, a ,adrenoceptor antagonist, in the macaque (9), and is reduced by atropine, a muscarinic receptor antagonist, as well as by prazosin, an a-1 adrenoceptor antagonist, in dogs (86). These data indicate that cholinergic and adrenergic pathways influence GHRP activity; however, in dogs, while metoxamine, an a-1 adrenoceptor agonist, does not modify the GH response to GHRP-6, contradictory results about the effect of clonidine, an a-2 adrenoceptor agonist, have been reported (78, 86).

The GH-releasing activity of GHRPs has also been found to be influenced by steroids (56, 75, 93). In rats, the GH response to GHRPs is increased by estradiol and testosterone and reduced by corticosteroids (56). The mechanisms underlying these influences are still unknown. On the other hand, in rats, the GH-releasing activity of GHRPs is inhibited by free fatty acids (56), whose action probably takes place at the pituitary level (94).

Studies addressing desensitization to the activity of compounds belonging to the GHRP family, both in vitro and in vivo, indicate that GHRPs and GHRH induce homologous but not heterologous desensitization (6, 31, 52, 53, 55, 57, 68, 70, 75). In spite of preserved pituitary GH releasable pool, clear desensitization to GHRP activity has been demonstrated during continuous GHRP infusion (31, 57, 68, 70, 75), as well as during frequent intermittent administration (57, 95). Interestingly, in rats, swine and dogs, this is not the case during less frequent intermittent GHRP administration up to 15 days (6, 29, 36, 75, 95, 96).

Prolonged GHRP administration in animals has already been shown to increase insulin-like growth factor I (IGF-I) levels (4, 29, 35, 36, 75, 77) indicating that GHRP-induced GH secretion is biologically active and that GHRP treatment is able to enhance the activity of the GH/IGF-I axis. In agreement with this assumption chronic treatment with GHRPs in rats has been shown to promote body growth or antagonize age-related changes in body composition and metabolism in animals (4, 76, 77). However, an intact hypothalamo-pituitary function is required for GHRP action (52, 56, 80, 81).

Both peptidyl and non-peptidyl GHRPs also possess slight prolactin (PRL), adrenocorticotropic (ACTH) and cortisol secreting activity (27–31, 34–36, 97). The mechanisms underlying these effects are still unclear. The stimulatory effect on PRL seems to include a direct effect on somatomammotropes cells (98). On the other hand, the stimulatory effect on cortisol seems to be due to the ACTH-releasing activity of GHRPs which, in turn, seems to be dependent on central mechanisms (56, 81, 99, 100). Interestingly, in rats GHRP-6 and corticotropin-releasing hormone (CRH) exhibit no interaction while GHRP-6 and arginine vasopressin (AVP) show a synergistic effect on ACTH secretion (97, 99). This evidence suggests a CRH-mediated action for GHRPs.

The stimulatory effect of GHRPs on the hypothalamic-pituitary-adrenal (HPA) axis is not negligible, however. In fact, chronic treatment with GHRPs in young, obese, diabetic rats worsens their diabetic and metabolic state probably by increasing the activity of the HPA axis (34).

Effects of GHRPs in humans

Physiological conditions

GHRP-6 was the first hexapeptide to be extensively studied in humans. More recently, the effects of other peptides, i.e. GHRH-1, GHRH-2 and Hexarelin (18, 20–26), and of non-peptidyl GHRPs (33, 37–41), have been studied in man.

The GH-releasing effect of GHRPs is dose-related (13–19, 101–103); the GH response to 1 μg/kg i.v. of these
peptides is generally higher than that elicited by 1 μg/kg i.v. GHRH (14, 18, 19, 103, 104), its maximal effective dose (105). Higher doses of i.v. GHRPs have rarely been studied but, recently, 2 μg/kg i.v. Hexarelin have been shown to elicit a further GH rise (103) (Fig. 2). Interestingly, unpublished results (E Arvat, L Di Vito, B Maccagno, F Broglio, MF Boghen, R Deghenghi, F Camanni & E Ghigo) demonstrated that 1 and 2 μg/kg Hexarelin and GHRP-2 have the same GH-releasing effect. Also non-peptidyl GHRPs have dose-related effects but their potency is clearly lower than that of peptides; in fact, the maximal effective i.v. dose of L-692.429 is probably greater than 1 mg/kg (33, 37, 38). A dose-related effect of GHRPs has also been shown after the subcutaneous, the intranasal and even the oral route of administration (15–18, 33, 103, 106) (Fig. 3).

Notably, the GH response to GHRPs shows good intra-individual reproducibility (103, 107), different from that observed with GHRH (108, 109).

The GHRP activity does not depend on sex (18, 103, 110) but it is age-dependent (18, 20, 26, 38, 111–115). The GH-releasing activity of Hexarelin is present at birth and persists at a similar level in prepubertal children. Then it clearly increases at puberty, persists at a similar level in young adults and then decreases in aging (26, 111–114) when, however, its effect is still higher than that of GHRH (111–113). Similar results have been observed studying the effect of GHRP-6, GHRP-1, GHRP-2, L-692.429 and MK-0677 in aging (18, 38, 40, 41, 115). The age-related effect of GHRPs is different from that of GHRH (Fig. 4) whose effect seems maximal at birth and then...
progressively decreases to very low activity in aging (26, 116, 117).

The mechanisms underlying the age-related activity of GHRPs are poorly understood. Some data suggest that the enhanced activity of GHRPs at puberty could depend on gonadal steroids. In fact, the GH response to Hexarelin is more marked in pubertal girls than in boys (114), is enhanced by testosterone (118) as well as by ethinyl-estradiol but not by oxandrolone (S Loche, P Cambiaso, D Carta, S Setzu, B P Imbimbo, P Borrelli, C Pintor & M Cappa, unpublished results) in children with constitutional delay of growth. While estradiol could play a role in the increased GH-releasing activity of GHRPs at puberty, the fall in estrogen levels during the menopause does not play a role in the reduction of the somatotrope response to GHRPs. In fact, 3-month treatment with transdermal estradiol does not modify the GH response to Hexarelin in postmenopausal women (119).

On the other hand, concomitant reduction of GHRH activity and somatostatinergic hyperactivity could play a role in the reduced effect of GHRPs in aging. In the elderly, the GH response to GHRPs is enhanced by both GHRH and arginine (111, 113), which acts via somatostatin inhibition (120), but only co-administration of these three substances restores the GH response to levels found in the young (26). Besides age-related variations in the neural control of somatotrope function, the possibility that the reduced GH-releasing activity of GHRPs in aging could, at least partially, depend on impairment of receptor or post-receptor mechanisms must also be considered. In fact, we found that the GH response to Hexarelin is improved, but not restored, by supramaximal doses of the hexapeptide (121).

To clarify the hypothalamo–pituitary mechanisms underlying the GH-releasing activity of GHRPs in man several studies have been performed in young adults. In man, as in animals (52, 53), the activity of GHRPs is not mediated by opioid receptors, naloxone being unable to modify the Hexarelin-induced GH response (122). GHRPs and GHRH have a synergistic effect (14, 18, 19, 61, 123–125) (Fig. 5) and even very low GHRP doses have been found to potentiate strikingly the GHRH-induced GH rise in man (14, 18, 123). These data agree with evidence in animals pointing to different mechanisms of action for these peptides (see above).

On the other hand, several studies have tried to clarify the interactions between GHRPs and neurotransmitters, metabolic fuels and hormones known to influence somatotrope function in man (Fig. 5).

While the GH-releasing effect of GHRP-6 is increased by pyridostigmine, a cholinergic agonist, or hypoglycemia but not by clonidine (19), the more marked GH stimulatory effect of Hexarelin is not modified by pyridostigmine, arginine or atenolol, a β-1 adrenergic antagonist, (61, 126), which are known to potentiate the GHRH-induced GH rise strongly. Notably, both pyridostigmine and arginine are unable to modify the synergistic effect of GHRPs and GHRH (127, 128). On the other hand, atropine and pirenzipine, a muscarinic M1 receptor antagonist, as well as salbutamol, a β-2 adrenergic agonist, which are known to nearly abolish the somatotrope response to GHRH via stimulation of hypothalamic somatostatin (120), only blunt the GHRP-induced GH response (19, 61, 126).

The GH response to GHRPs, namely Hexarelin, GHRP-6 and L-692,429, is also partially resistant to inhibition by other substances known to abolish the GHRH-induced GH rise. The GH response to GHRPs is only blunted by glucose load, glucocorticoids and recombinant human (rh) GH, which probably stimulate hypothalamic somatostatin release (62, 128–131). Similarly, the increase in circulating free fatty acids, which could act directly at the pituitary level antagonizing depolarization of somatotrope cell membrane (94), only blunts the GH response to GHRPs (62) which, on the other hand, is enhanced by acipimox, a lipolysis inhibitor (132) (Fig. 5).

Even the infusion of exogenous somatostatin, at a dose able to abolish the GHRH-induced GH rise, inhibits but does not abolish the somatotrope responsiveness to Hexarelin (61).

Alltogether, these findings in man agree with others in animals favoring the hypothesis that mechanisms underlying the GH-releasing activity of GHRPs include antagonization of somatostatinergic activity both at the pituitary and the hypothalamic level (54, 79). This may also explain the good intra-individual reproducibility of the GH response to GHRPs (103, 107).

Interestingly, to emphasize that the activity of GHRPs mainly depends on functional hypothalamic integrity, it has been shown that the GH-releasing effect of GHRP-6 and Hexarelin, either alone or in combination with GHRH, is strongly reduced in patients with pituitary stalk disconnection (63, 133, 134). Moreover, the reduced effect of Hexarelin in aging is fully restored to
Figure 5 Neuroendocrinological and metabolic influences on the GH-releasing effect of Hexarelin (HEX) and/or GHRH in young adults. OGTT, oral glucose tolerance test; FFA, free fatty acids; SRIF, somatostatin.
The existence of young continuous homologous GHRPs that could partially release GH has already been shown in the literature or still hypothetical. +, stimulatory effect; −, inhibitory effect; SRIF, somatostatin.

![Diagram of GH secretion control during the human lifespan](image)

Figure 6: Neural control of GH secretion during the human lifespan (dotted lines in 'elderly' indicate reduced activity of the neural pathways, already shown in the literature or still hypothetical). +, stimulatory effect; −, inhibitory effect; SRIF, somatostatin.

Young levels only when it is co-administered with GHRH and arginine (26). These data confirm that the releasable GH pool in the pituitary does not vary with age (116) and reinforce the hypothesis that, at least partially, the activity of GHRPs is independent of both GHRH and somatostatin activity. This points to the existence of an unknown (U) endogenous factor which could mediate the effects of GHRPs (18, 52) and implies that U factor activity could be impaired in aging (Fig. 6).

As in animals, in humans there is evidence that GHRPs and GHRH induce homologous but not heterologous desensitization (101, 104, 107, 125, 135–137). The homologous desensitization has been shown during continuous GHRP infusion (101, 107, 135); moreover, at the end of GHRP infusion the GH response to an acute hexapeptide challenge is reduced (101, 107, 135). On the other hand, according to data in animals (28, 75, 95, 96), desensitization does not seem to occur during intermittent administration. In fact, the GH response to acute GHRP-6 or Hexarelin is preserved after oral or intranasal treatment (administered thrice daily) up to 15 days (112, 138).

It has been shown that prolonged GHRP administration by parenteral, intranasal and oral administration enhances spontaneous GH pulsatility over 24 h and increases IGF-I levels in normal young adults as well as in short children and even in elderly subjects (15, 39–41, 107, 135, 138, 139), indicating that treatment with GHRPs is able to augment the activity of the GH/IGF-I axis.

The activity of GHRPs is not fully specific for GH release: in fact, slight but significant and dose-dependent PRL-, ACTH- and cortisol-releasing activity has been demonstrated for all peptides and non-peptidyl compounds (13, 14, 18, 37–39, 102, 103, 129, 140). Moreover, a thyrotropin (TSH)-inhibiting effect has been reported for GHRPs (135, 141). The mechanisms underlying these effects are unknown. Until now, it has only been demonstrated that, in man, the PRL- and ACTH/cortisol-releasing activities of Hexarelin are not
modified by naloxone (122), cyproheptadine, a serotoninergic antagonist, or diphenhydramine, an histaminergic antagonist (142). Thus, these releasing activities of GHRPs do not depend on mediation by opioids, serotonin or histamine which are known to play important roles in the control of PRL and ACTH secretion (85). On the other hand, our preliminary results in normal men indicate that, in contrast to rats, Hexarelin does not show any interaction with either hCRH or AVP on ACTH secretion. Interestingly, the hormonal activities of GHRPs show different age-related patterns. In fact, in aging the stimulatory effect of Hexarelin on GH secretion is reduced, that on PRL is unchanged and that on ACTH is increased (143).

**Pathological conditions**

The GH-releasing activity of GHRPs has also been tested in some human pathophysiological conditions with particular attention to GH hyper- and hypothalamic secretory states.

Activity is preserved in the presence of somatotrope adenomas both in vitro (98, 144, 145) and in vivo (146–150). In vitro GHRP-6 releases more GH than GHRH from somatotropinoma cells and is active even on cells refractory to GHRH stimulation (98, 144, 145, 151). Moreover, the mean somatotrope responsiveness to GHRP-6 both alone and combined with GHRH in acromegalic patients is similar to that in normal subjects, though a dramatic inter-individual variability is present (146). In acromegalis bearing a somatotropinoma adenoma, both GH and PRL responses to Hexarelin have been found similar to those in normal subjects (150). Interestingly, this is not the case in patients with idiopathic hyperprolactinemia, in whom Hexarelin is unable to modify high PRL levels while eliciting a GH response clearly lower than that in controls (150). Thus, it appears that hyperprolactinemic patients are partially refractory to the activity of GHRPs.

A preserved GH-releasing effect of GHRPs has also been reported in patients with functional GH hyposecretory states such as anorexia nervosa (152) as well as in hypothyroid patients (153). Interestingly, while in anorexia the GH response to GHRH has also been shown to be exaggerated (154), that in hyperthyroidism is usually reduced (153, 155).

On the other hand, GHRPs alone and in combination with GHRH have been found to induce marked GH responses in children with idiopathic short stature (106, 114, 118, 133, 141, 156–158) and in patients with GH deficiency either in childhood or in adulthood (63, 133, 134, 156–162). The possible usefulness of GHRPs to test the maximal secretory capacity of somatotrope cells in the diagnosis of GH deficiency has been hypothesized by some (106, 118, 133, 141, 160) but not by others (114, 157, 161) authors. Notably, in children as well as in adults with pituitary stalk lesion (63, 133, 134) the GH responses to GHRPs alone and combined with GHRH are greatly impaired.

The possible therapeutic usefulness of GHRPs in children with short stature has also been tested (139, 163–166). Recent data demonstrate that, in open studies, chronic treatment with i.n. Hexarelin in children with idiopathic short stature and s.c. GHRP-2 in children with GH insufficiency is able to increase IGF-I levels and growth velocity (139, 164, 165).

During prolonged treatment with MK-0677, a clear IGF-I increase has been found even in adult patients with GH deficiency in spite of a small increase in spontaneous GH secretion (162).

GHRPs alone and combined with GHRH have been shown to elicit marked GH release in obese patients in whom there is a well known reduction in somatotrope function (118, 123, 127, 167–169). The GH response to GHRPs in obesity is marked but is still lower than in controls (127, 167–169); noteworthy, inhibition of lipolysis by acipimox is able to enhance further the GH response to the combined administration of GHRP-6 and GHRH in obese patients (168), further pointing to the role of metabolic alterations in the pathogenesis of GH insufficiency in obesity.

The GH-releasing effect of GHRP-6 both alone and combined with GHRH is greatly reduced in hypothyroidism (170, R Valcavi, personal communication) and nearly absent in Cushing’s syndrome (171, 172), pointing to the severity of GH insufficiency in this latter condition. On the other hand, in catabolic critically ill adults a strong GH-releasing activity of GHRPs has recently been reported (173).

**Clinical perspectives for GHRPs**

Great attention has been given to GH secretagogues, such as GHRPs, in considering their potent GH-releasing effect as well as their activity after the oral route of administration and looking at their possible usefulness in enhancing the activity of the GH/IGF-I axis. Here, we briefly consider possible targets for GHRPs in clinical practice.

**Diagnosis of GH deficiency**

Both in childhood and in adulthood, it is still a matter of debate what is the best test for the diagnosis of GH deficiency. In particular, classical provocative tests have been found to be unreliable, as has GHRH, at least when given alone (174). The potent GH-releasing activity of GHRPs given alone or, even better, in combination with GHRH, suggest that these peptides could be used as provocative stimuli to assess the pituitary GH-releasable pool. Some data already reinforce (106, 118, 133, 141, 160) while others reduce (114, 157, 161) this hypothesis. Anyway, as the effect of GHRPs is dependent on age (18, 20, 26, 38, 111–113), age-related normative values of GH response to these peptides
have to be defined. Moreover, it remains clear that, at least in childhood, a subject showing normal GH response to a provocative stimulus may present with impaired spontaneous GH secretion over 24h (175, 176).

Treatment of GH deficiency and short stature

Although some promising preliminary results have been reported in children treated with GHRPs for up to 6 months (139, 165), their efficacy must be verified in double-blind, placebo-controlled studies.

Theoretically, GHRPs are unlikely to be therapeutically useful in patients with GH deficiency due to hypopituitarism: this applies not only to GH deficiency in childhood but also in adulthood when only severe somatotrope deficiency seems to cause clear alteration in body composition, structure functions and metabolism (177, 178).

On the other hand, the possibility that GHRP treatment could usefully enhance the activity of the GH/IGF-I axis in short children with GH deficiency due to hypothalamic alterations is more likely, a notable exception being patients bearing pituitary stalk disconnection (63, 133, 134). Finally, GHRPs could be useful for treatment of idiopathic short stature but it has to be remembered that even treatment with rhGH often gives poor results (179).

Aging

It is still unclear whether it is really useful to restore the activity of the GH/IGF-I axis in aging (116, 180, 181). However, as the pituitary GH releasable pool is preserved in elderly subjects (116), GH secretagogues are probably the most appropriate approach in restoring GH and IGF-I secretion in order to counteract age-related changes in body composition, structure function and metabolism, with particular attention to the effects on muscle mass and strength, bone mass and atherogenesis (116, 180, 181). Actually, in elderly subjects short term oral GHRP treatment does not seem to undergo desensitization and shows a tendency to increase IGF-I levels (40, 112, 138). Thus, the possible usefulness of GHRPs has to be verified in chronic, double-blind, placebo-controlled studies.

Catabolic states and dilated cardiomyopathy

Theoretically, it is unlikely that peripheral GH resistance in severe catabolic states (182) would be overridden by GHRP-induced increase of somatotrope secretion: in fact, in these conditions even very high rhGH doses have still to be clearly shown to be useful. On the other hand, GHRPs could be useful in increasing the activity of the GH/IGF-I axis and inducing anabolism in mild catabolic states or in the phase of recovery from severe catabolism (140).

Based on recent data reporting the possible usefulness of treatment with rhGH on cardiac function in patients with idiopathic dilative cardiomyopathy in the absence of GH deficiency (183), the possible usefulness of GHRP-induced increase in the GH/IGF-I axis activity in this pathophysiological condition has also to be verified.

Conclusions

About 20 years after their discovery GHRPs are a hot topic in neuroendocrinology; in fact biologists, clinicians and pharmaceutical companies are actively involved in this field. However, 20 years later, we still do not know what are the exact mechanisms underlying the activity of these synthetic, non-natural peptides which possess strong GH- but also significant PRL- and ACTH-releasing activity. The existence of an endogenous GHRP-like ligand is a fascinating possibility and, after the GHRP receptor has been cloned, we are probably approaching its discovery. However, we should not necessarily wait for an endogenous peptide whose main action is stimulation of GH secretion. As suggested by a large number of studies, the most important conceptual discovery about GHRPs could be that their activity depends on interactions with several factors rather than on their own potency. This is not simply phylosophy.

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