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Growth Hormone-Releasing Peptides and Their Analogs

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Growth hormone-releasing peptides (GHRPs) are a series of hepta (GHRP-1)- and hexapeptides (GHRP-2, GHRP-6, Hexarelin) that have been shown to be effective releasers of GH in animals and humans. More recently, a series of nonpeptidyl GH secretagogues (L-692,429, L-692,585, MK-0677) were discovered using GHRP-6 as a template. Some cyclic peptides as well as penta-, tetra-, and pseudotripeptides have also been described. This review summarizes recent developments in our understanding of the GHRPs, as well as the current nonpeptide pharmacologic analogs. GHRPs and their analogs have no structural homology with GHRH and act via specific receptors present at either the pituitary or the hypothalamic level. The GHRP receptor has recently been cloned and 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. Although the exact mechanism of action of GHRPs has not been fully established, there is probably a dual site of action on both the pituitary and the hypothalamus, possibly involving regulatory factors in addition to GHRH and somatostatin. Moreover, the possibility that GHRPs act via an unknown hypothalamic factor (U factor) is still open. The marked GH-releasing activity of GHRPs is reproducible and dose-related after intravenous, subcutaneous, intranasal, and even oral administration. The GH-releasing effect of GHRPs is the same in both sexes, but undergoes age-related variations. It increases from birth to puberty and decreases in aging. The GH-releasing activity of GHRPs is synergistic with that of GHRH and not affected by opioid receptor antagonists, while it is only blunted by inhibitory influences that are known to nearly abolish the effect of GHRH, such as neurotransmitters, glucose, free fatty acids, glucocorticoids, rhGH, and even exogenous somatostatin. GHRPs maintain their GH-releasing effect in somatotrope hypersecretory states, such as acromegaly, anorexia nervosa, and hyperthyroidism. On the other hand, GHRPs and their analogs have been reported to be effective in idiopathic short stature, in some situations of GH deficiency, in obesity, and in hypothyroidism, while in patients with pituitary stalk disconnection and in Cushing's syndrome the somatotrope responsiveness to GHRPs is almost absent. A potential role in the treatment of short stature, aging, catabolic states, and dilated cardiomyopathy has been envisaged. KEY WORDS: GH; GHRP; GH secretagogues; GH deficiency; aging; catabolic states.

INTRODUCTION

Growth hormone-releasing peptides (GHRPs) are a series of hepta (GHRP-1)- and hexapeptides (GHRP-2, GHRP-6, Hexarelin) that have been shown to

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be effective releasers of GH in animal and man, after intravenous, subcutaneous, intranasal, and even oral administration (21, 25, 26, 53, 54, 61, 103, 106, 124). The first GHRP identified, GHRP-6, was derived from the pentapeptide met-enkephalin through theoretic low-energy conformational calculations, computer modeling, and structural modification (128). Although GHRPs were based on an opioid peptide, they were devoid of opioid activity and the modifications required to induce the pituitary GH-releasing activity did not appear to be dependent on opiate receptors (44). More recently, a series of nonpeptidyl GH secretagogues (L-692,429, L-692,585, MK-0677) were discovered using GHRP-6 as a template. Some cyclic peptides as well as penta-, tetra-, and pseudotripeptides were also described (31, 38, 55–57, 64, 71, 91, 100, 125, 132, 158) (Fig. 1).

Although the exact mechanism of action of GHRPs and their analogs has not been fully established, there is probably a dual site of action on both the pituitary and the hypothalamus, where these agents have their own specific, non-GHRH, nonsomatostatin, non-opioid receptors (see below). This evidence suggests the existence of a still unidentified endogenous GHRP-like ligand.

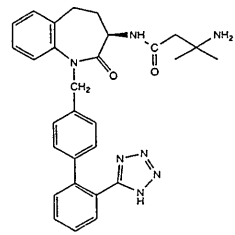
GHRP RECEPTORS AND THE SIGNAL TRANSDUCTION PATHWAY

A wealth of data indicate that the actions of GHRPs and nonpeptidyl GHRP mimetics are mediated by specific receptors and intracellular mechanisms distinct from those of GHRH. The GHRP receptor has recently been cloned. The full-length human and swine receptor cDNAs encoded a predicted polypeptide of 366 amino acids with seven transmembrane domains belonging to 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 (94).

A specific high-affinity binding site that mediates the activity of both GHRPs and nonpeptidyl GHRP mimetics has been identified in anterior pituitary and hypothalamic membranes of rat and pig (45, 141, 156, 167). The binding affinity of these structurally different GH secretagogues to the same receptor is correlated with their GH stimulatory effect (141). The binding is Mg^{2+}-dependent and inhibited by stable GTP analogs and is not displaced by GHRH, somatostatin, metenkephalin, substance P, galanin, GnRH, TRH, PACAP-38, gastrin-releasing peptide, MSH, and other neurotransmitters (45, 141, 156, 167).

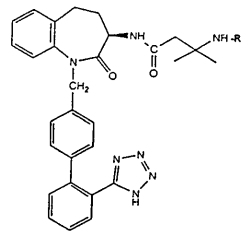
Recently our group identified the presence of GHRP receptors in humans (129) in whom these compounds have been shown to have the highest GH-releasing effect (25, 26, 79, 103). Among various tissues tested "postmortem," the hypothalamus and the pituitary gland of adult subjects showed the highest specific GHRP binding. Well detectable specific GHRP binding was also found in choroid plexus and cerebral cortex but not in the cerebellum and "corpus callosum" (Fig 2). No significant sex-related difference in GHRP binding to various tissues was found. The GHRP receptors detected in human brain and pituitary gland have basic binding properties that conform to those described in

1977	Tyr-D-Trp-Gly-Phe-Met-NH_2	(D-Trp^2)MetEKNH_2
1981	Tyr-D-Trp-Ala-Trp-D-Phe-NH_2	
1984	His-D-Trp-Ala-Trp-D-Phe-NH_2	
	His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2	GHRP-6
1991	Ala-His-DβNal-Ala-Trp-D-Phe-Lys-NH_2	GHRP-1
1992	His-D2MeTrp-Ala-Trp-D-Phe-Lys-NH_2	Hexarelin



L-692,429

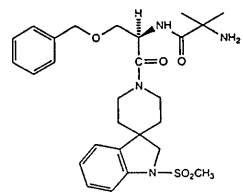
1993	D-Ala-DβNal-Ala-Trp-D-Phe-Lys-NH_2	GHRP-2
1994		



L-692,585

R = -CH_2CHOH-CH_3

1995	INIP-DβNal-D-Trp-D-Phe-Lys-NH_2	G 7509
	INIP-DβNal-DβNal-Phe-Lys-NH_2	G 7039



MK-0677

	GAB-D-2MeTrp-DβNal-Phe-Lys-NH_2	EP 50885
	GAB-D-2MeTrp-D-2MeTrp-2MeTrp-Lys-NH_2	EP 51216
1996	IMPR-D-Phe-Ala-Trp-D-Phe(CH2NH)-Lys-ol	NNC26-0194
	Aib-D-2MeTrp-D-2MeTrp-NH_2	EP 51389

FIG. 1. Chronology and structure of various peptidyl and nonpeptidyl GH secretagogues.

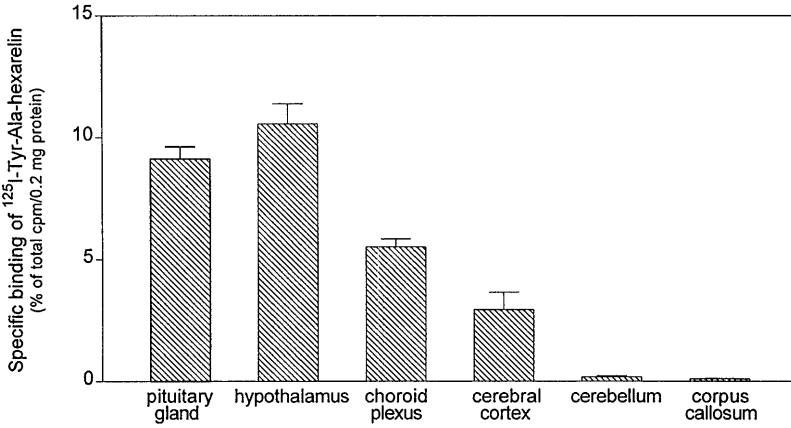


FIG. 2. Specific binding of ^{125}I-labeled Tyr-Ala-Hexarelin to membranes from pituitary gland and different brain regions of adult male subjects. Specific binding was calculated as the difference between binding in the absence and the presence of excess unlabeled Tyr-Ala-Hexarelin. Values represent means ± SD of four subjects.

rat and pig hypothalamus and pituitary gland (45, 156, 167). In fact, we have found that different GHRPs, 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, and galanin (129).

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 (14, 15, 23, 37, 42, 46, 58, 84, 86, 119, 152) and in humans (7, 22, 118, 134, 143) 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 some "nonneuroendocrine" CNS functions. In fact, it has been shown in rodents that parenteral and intracerebroventricular GHRP administration stimulates feeding behavior and C-Fos mRNA expression in the arcuate nucleus (58, 98, 117, 157). Moreover, in humans, parenteral GHRP injection has been reported to alter sleep patterns (3, 69). All together, 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 that could be involved in neuroendocrine and extraneuroendocrine activities.

In agreement with evidence that GHRPs and GHRH possess distinct specific receptors, these peptides display different signal transduction pathways (2, 37, 84, 152, 171). In fact, whereas GHRH activates protein kinase A via stimulatory effects on adenyl cyclase and cAMP production (37), GHRPs and nonpeptidyl GHRP mimetics, with the notable exception of GHRP-2 (170), act via intracellular mechanisms not including cAMP synthesis (2, 37, 39, 171). GHRPs have been reported to increase intracellular Ca^{2+} and to activate ionic conductance

involved in producing cell membrane depolarization (2, 37, 84, 90, 152, 171). This effect is blocked by chelation of extracellular calcium or by using calcium channel blockers (2, 39, 90, 152). GHRP-induced activation of G protein pathways coupled to potassium channels and phospholipase C has been shown in pituitary cell cultures (141).

EFFECTS OF GHRPs AND THEIR ANALOGS IN ANIMALS: IN VITRO AND IN VIVO STUDIES

These agents have been found to be potent GH secretagogues but also possess significant ACTH- and PRL-releasing activity.

GH-Releasing Effect

GHRPs and their analogs stimulate GH secretion *in vitro* and *in vivo* in a number of different species.

In vitro GHRPs and nonpeptidyl GHRP mimetics stimulate GH release from somatotrope cells (2, 14, 23, 37–39, 84, 90, 114, 152, 158, 170, 171), probably augmenting the number of GH-secreting cells more than increasing the amount of GH secreted per cell (84). Moreover, GHRPs release GH from both sparsely granulated and heavily granulated rat pituitary cells (103). Interestingly, GHRPs have been reported to induce GH synthesis (114).

In vitro the GH-releasing activity of GHRPs is lower than that of GHRH (14, 15, 23, 152). Under this condition, an additive or a true synergistic effect of GHRPs on GHRH-stimulated GH has been reported (14, 23, 37, 39, 170, 171). The stimulatory effect of GHRPs on GH secretion from somatotrope cells is abolished by specific GHRP, but not by GHRH antagonists (23, 37, 84). However, GHRP-2 is an exception (170). Although somatostatin inhibits the stimulatory effect of GHRPs on GH secretion from pituitary (2, 14, 15, 21), there is evidence suggesting that GHRPs could act by antagonizing the inhibitory activity of somatostatin on GH release by counteracting its hyperpolarizing effect on somatotrope cell membranes (84).

It has to be pointed out that the GH-releasing activity of GHRPs is clearly higher in hypothalamic–pituitary preparations than in pituitary preparations (123). This finding may explain why the stimulatory effect of GHRPs on GH secretion is greater *in vivo* than *in vitro* (23, 42). *In vivo*, GHRPs show synergistic effects on GHRH-stimulated GH release (23, 120) and prevent the normal cyclic refractoriness to GHRH (42). Moreover, the activity of GHRPs is age-dependent, with a decrease in aged animals (30, 153, 168, 169). These data, as well as evidence that the GH response to direct intracerebroventricular GHRP injection is greater than that observed after systemic administration of the same dose (65), point to an important hypothalamic action of GHRPs. In animals with lesions of the pituitary stalk, the GH-releasing effect of GHRP-6 and L-692,585 and their synergism with GHRH are reduced, although not

abolished (68, 92, 119). Moreover, hypothalamic ablation initially increases and subsequently reduces GHRP activity (119), indicating that normal hypothalamic–pituitary function is needed for full GHRP activity.

The possibility that hypothalamic GHRP activity is mediated by 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* (23, 37, 38, 120) does not rule out the possibility that the GH-releasing effect of GHRPs is partially mediated by GHRH. Although there are data against a GHRH-mediated action (114), many results indicate that the integrity of GHRH function is needed for the full GH-releasing effect of GHRPs. In fact, passive immunization against GHRH, as well as pretreatment with GHRH antagonist, reduces the activity of GHRPs (19, 23, 42, 46, 172). An increased release of GHRH in hypophysial portal blood after GHRP administration has also been shown in sheep (86). GHRPs are active in dwarf mice (103) but not in the lit/lit mouse, which has no pituitary GHRH receptors (59). Moreover, both in GH-deficient rats and in lit/lit mice, systemic administration of GHRPs and their analogs activates a subpopulation of hypothalamic arcuate neurons where increased electrical activity, cFos-like immunoreactivity, and c-Fos mRNA expression have been found (58, 157). Noteworthy, it is well known that in the arcuate nucleus the highest density of GHRH-secreting neurons is present (130).

At the hypothalamic level, GHRPs do not seem to negatively influence somatostatin secretion; the release of somatostatin from hypothalamic preparations is not reduced by GHRPs and their analogs (105). Passive immunization against somatostatin does not reduce the GH-releasing effect of GHRPs (23, 46, 114, 172), while it abolishes that of cholinergic agonists (130). Moreover, GHRPs and substances known to inhibit hypothalamic somatostatin release have been reported to have synergistic effects on GH secretion (120, 131). On the other hand, it has been hypothesized that the positive influence of GHRPs on GHRH-secreting neurons could be due to functional antagonism to the inhibitory influence of somatostatin at this level. In rats, the GH stimulatory effect of intracerebroventricular administration of GHRPs, but not that of intravenous (iv) GHRH, is blocked by intracerebroventricular pretreatment with octreotide (65). These data suggest that GHRPs could act as functional somatostatin antagonists at the hypothalamic as well as at the pituitary level (84).

It has recently been reported in swine that the infusion of a GHRP antagonist inhibits not only the GH response to acute GHRP administration, but also the spontaneous GH pulsatility (32). If confirmed, this result would strengthen the hypothesis that a natural GHRP-like ligand plays a major role in the physiological control of GH secretion.

The interaction of GHRPs with neurotransmitters and neuropeptides has also been studied. In rats, the GH-releasing effect of GHRP-6 is not modified by naloxone, while it is synergistic with that of dermorphin and metenkephalin analogs (23). This suggests 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 GHRP-6 as well as of GHRH (159). Also substance P could mediate the activity of GHRPs, while bombesin could play an inhibitory role in rats (20, 119). The GH response to GHRP-6 is increased by pyridostigmine (131), a cholinesterase inhibitor, and by propranolol (120), a β-adrenoceptor antagonist, whereas it is reduced by atropine, a muscarinic receptor antagonist, and by prazosin (131), an α_1-adrenoceptor antagonist. These data seem to indicate that cholinergic and adrenergic pathways influence GHRP activity in animals. However, metoxamine, an α_1-adrenoceptor agonist, does not modify the GH response to GHRP-6 in the dog, while contradictory results about the effect of clonidine, an α_2-adrenoceptor agonist, have been reported (30, 131).

The GH-releasing activity of GHRPs has also been found to be influenced by steroids (18, 119, 153). In rats, the GH response to GHRP-6 is increased by etradiol and testosterone and reduced by corticosteroids (119). The mechanisms underlying these influences are still unknown. On the other hand, in rats, the GH-releasing activity of GHRP-6 is inhibited by free fatty acids (119), whose action likely takes place at the pituitary level (29).

In vitro and *in vivo*, GHRPs and their analogs as well as GHRH induce homologous but not heterologous desensitization (14, 15, 23, 31, 37, 42, 153, 170, 171). Despite a preserved pituitary GH releasable pool, desensitization to GHRP activity has been demonstrated during infusion of GHRPs and nonpeptidyl GHRP mimetics (31, 42, 153, 170, 171), as well as after frequent intermittent administration of these compounds (42, 66). Interestingly, this does not occur after less frequent daily intermittent GHRP administration for up to 15 days (14, 62, 66, 99, 153).

Prolonged administration of GHRPs and their analogs in animals increases IGF-I levels (21, 93, 99, 100, 153, 169), indicating that GHRP-stimulated GH 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 and antagonize age-related changes in body composition and metabolism (21, 168, 169). However, an intact pituitary function is required for GHRP action (23, 68, 92, 119).

Recently newly synthesized small size GHRPs have been described, some of which were found to be more potent than GHRP-6 (55).

ACTH- and PRL-Releasing Effect

Both GHRPs and nonpeptidyl GHRP mimetics also possess slight PRL-, ACTH-, and cortisol-stimulating activity (31, 38, 43, 91, 93, 99, 100, 158, 160). The mechanisms underlying these effects are still unclear. The stimulatory effect on PRL secretion seems to include a direct effect on somatomammotrope cells (1). The stimulatory effect on cortisol is due to the ACTH-releasing activity of GHRPs, which, in turn, seems to be dependent on central mechanisms (92, 119, 149, 155). In fact, GHRPs have been reported to stimulate GH but not ACTH secretion from rat pituitary (38, 64). On the other hand, the stimulatory

effect of GHRPs on hypothalamo–pituitary–adrenal (HPA) axis is reduced in rat with hypothalamo–pituitary disconnection (92). Interestingly, in rats GHRP-6 and CRH have no interaction, while GHRP-6 and arginine vasopressin (AVP) show a synergistic effect on ACTH secretion (149, 160). This suggests a CRH-mediated action for GHRPs. Moreover, the stimulatory effect of GHRPs and their analogs on HPA axis is not negligible. Chronic treatment with these compounds in obese diabetic rats worsens their metabolic state, probably by increasing the activity of the HPA axis (43).

EFFECTS OF GHRPs AND THEIR ANALOGS IN HUMANS

Physiological Conditions

GHRP-6 is the first hexapeptide that has been extensively studied in humans. More recently, the effects of other peptides, i.e., GHRP-1, GHRP-2, and Hexarelin (25, 26, 40, 53, 55, 79, 103, 109) and of a nonpeptidyl GHRP mimetic, i.e., L-692,429 (3, 33, 34, 47, 57, 71), have also been studied.

GH-Releasing Effect

The GH-releasing effect of GHRPs is dose-related (22, 52, 75, 96, 97) (Fig. 3). The GH-release induced by 1 μg/mg iv GHRP is greater than that elicited by 1 μg/kg iv GHRH (22, 26, 75, 148), which has been shown to be the maximal effective dose (70). Higher doses of iv GHRPs have rarely been studied, but we showed that 2 μg/kg iv Hexarelin is able to elicit a further increase in GH levels compared to the 1 μg/kg dose (75). However, our recent results (13) demonstrate

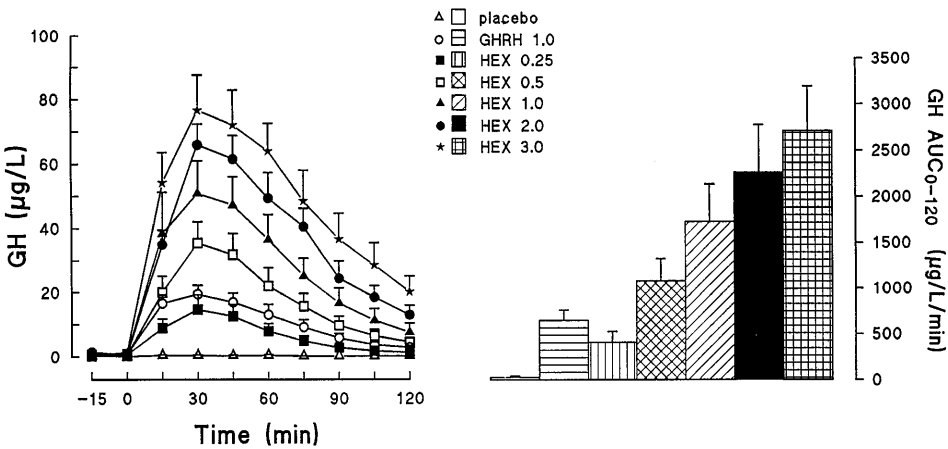


FIG. 3. Mean (±SEM) GH response curves (left) and AUCs (area under the curves, right) after placebo, 1 μg/kg iv GHRH, and 0.25, 0.5, 1.0, 2.0, and 3.0 μg/kg iv Hexarelin (HEX) in young adult humans.

that 1 and 2 μg/kg Hexarelin and GHRP-2 have the same GH-releasing effect (Fig. 4). A dose-related effect of GHRPs has also been shown after subcutaneous, intranasal, and oral administration (24, 26, 57, 75, 88, 89, 108). Nonpeptidyl GHRP mimetics also have dose-related effects but their potency is clearly lower than that of the peptides; for example, the maximal effective iv dose of L-692,429 is probably greater than 1 mg/kg (3, 57, 71). Notably, the GH response to GHRPs shows good intraindividual reproducibility (70, 75), which is not what is observed with GHRH (123, 165).

As noted above, the activity of GHRP and its analogs does not depend on gender (27, 75, 135), while it is age-dependent (3, 6, 16, 25, 26, 76, 77, 79, 127). The GH-releasing activity of Hexarelin recorded at birth persists unchanged until prepuberty; then, it clearly increases at puberty and decreases in aging (6, 16, 76, 77, 79) (Fig. 5). However, in aging the GH-releasing effect of Hexarelin is still higher than that of GHRH (6, 76, 77). Similar results have been observed in aging studying the effect of GHRP-6, GHRP-1, GHRP-2, and L-692,429 (3, 26, 33, 127). The pattern of the age-related GH-releasing effect of GHRPs is different from that of GHRH, the effect of which seems maximal at birth and then progressively decreases into old age (36, 74, 79) (Fig. 5).

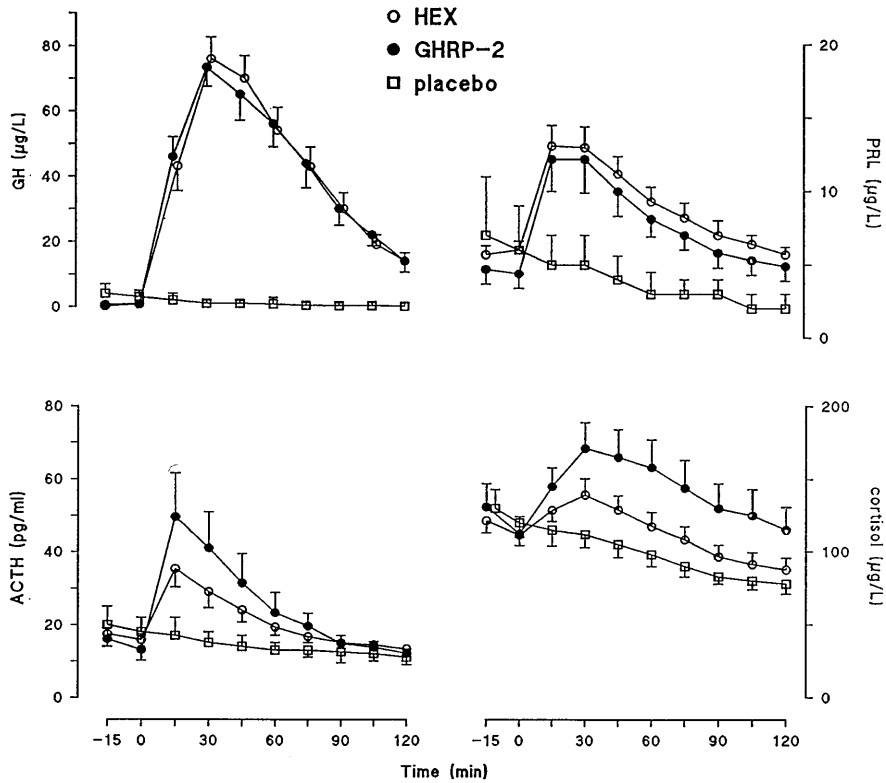


FIG. 4. Mean (±SEM) GH, PRL, ACTH, and cortisol responses to Hexarelin (HEX, 2.0 μg/kg iv), GHRP-2 (2.0 μg/kg iv), and placebo in six young adults.

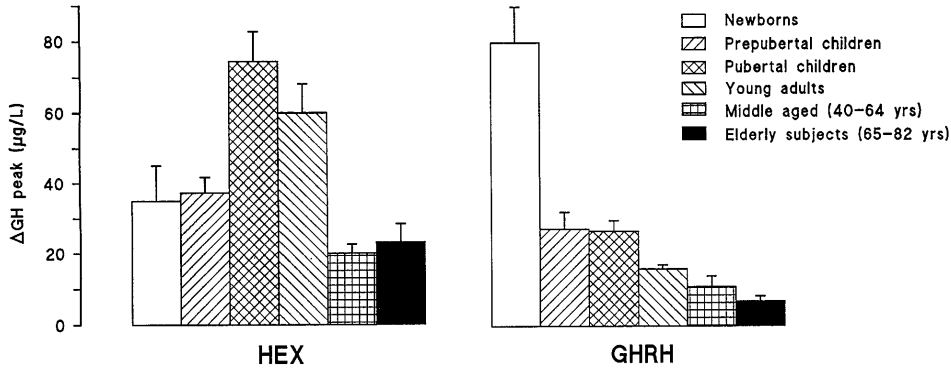


FIG. 5. Mean (±SEM) GH peak increment above baseline (ΔGH peak) after Hexarelin (HEX, 2 μg/kg iv) and GHRH (1 μg/kg iv) administration during lifespan.

The mechanisms underlying the age-related GH-releasing effect 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 at puberty is more marked in girls than in boys, and it is positively related to estradiol levels (16). Moreover, it is enhanced by testosterone as well as by ethynyl-estradiol in children with constitutional growth delay (116). While estradiol could play a major role in increasing the GH-releasing activity of GHRPs at puberty, the fall of estrogen levels in menopause does not seem to play a role in reducing the somatotrope response to GHRPs. In fact, in postmenopausal women 3-month treatment with transdermal estradiol (delivery 50 μg/die) increased serum estradiol levels, making them similar to those detected in young women but did not modify the reduced GH-releasing effect of Hexarelin (11). Concomitant reduction in GHRH release and increased somatostatinergic hypothalamic tone could play a role in reducing the GH-releasing effect of GHRPs in aging. In this condition the GH response to Hexarelin is enhanced by both GHRH and arginine (6, 76), but only coadministration of the three substances restores the GH response to young levels (79). It has to be pointed out that arginine probably acts via inhibition of hypothalamic somatostatin release (73). In addition to age-related variations in the neural control of somatotrope secretion, it is possible that the reduced GH-releasing activity of GHRPs in aging could depend in part on impairment of receptor or postreceptor mechanisms. We found that the GH response to Hexarelin is improved, although not restored, by supramaximal doses of the hexapeptide (9).

To clarify the hypothalamo–pituitary mechanisms underlying the GH-releasing activity of GHRPs in humans, several studies have been performed in young adult volunteers. As in animals (23, 37), the activity of GHRPs is not mediated by opioid receptors; naloxone is unable to modify the Hexarelin-induced release of GH (104). GHRPs act synergistically with GHRH (7, 22, 26, 27, 82, 122, 134) and even a very low GHRP dose has been found to enhance the GHRH-induced GH rise in man (22, 26, 27). These data agree with evidence in

animals pointing to different mechanisms of action for GHRPs and GHRH (see above).

Other studies tried to clarify the interactions between GHRPs and neurotransmitters, metabolic fuels and hormones known to influence somatotrope secretion in humans (Fig. 6). While the GH-releasing effect of GHRP-6 has been found to be increased by pyridostigmine, a cholinergic agonist, or hypoglycemia, but not by clonidine (134), the more marked GH stimulatory effect of Hexarelin is not modified by pyridostigmine, arginine, and atenolol (7, 10). These drugs are able to strongly potentiate the GHRH-induced GH response, probably acting via inhibition of hypothalamic somatostatin release (73). Both pyridostigmine and arginine are also unable to modify the synergistic effect of GHRPs on GHRH-stimulated GH release (8, 49). On the other hand, pirenzepine, a muscarinic M1 receptor antagonist, and salbutamol, a β_2-adrenergic agonist, which are known to nearly abolish the somatotrope response to GHRH via stimulation of hypothalamic somatostatin release (73), only blunt the Hexarelin-induced GH rise (7, 10). Similarly, the somatotrope response to coadministration of GHRP-6 and GHRH is blunted only by atropine (134). The

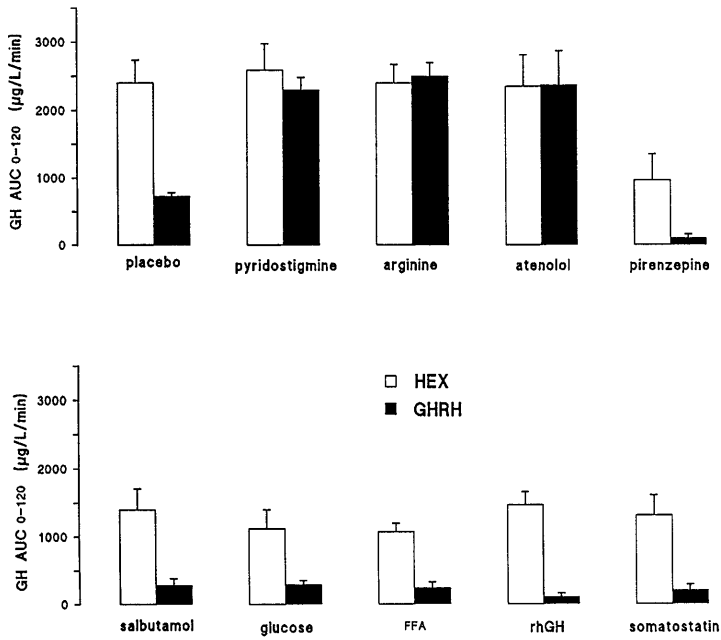


FIG. 6. Neuroendocrinological and metabolic influences on the GH-releasing effect of Hexarelin (HEX, 2 µg/kg iv) or GHRH (2 µg/kg iv) in young adults. FFA, free fatty acids; rhGH, recombinant human GH. Hex vs GHRH = $p < 0.01$. The GH response to Hex was not modified by pyridostigmine, arginine, or atenolol, while it was reduced by pirenzepine ($p < 0.05$), salbutamol ($p < 0.03$), glucose ($p < 0.05$), FFA ($p < 0.05$), rhGH ($p < 0.04$), and somatostatin ($p < 0.05$). The GH response to GHRH was increased by piridostigmine ($p < 0.01$), arginine ($p < 0.01$), and atenolol ($p < 0.01$), and was abolished by pirenzepine ($p < 0.01$), salbutamol ($p < 0.01$), glucose ($p < 0.01$), FFA ($p < 0.01$), rhGH ($p < 0.02$), and somatostatin ($p < 0.01$).

GH response to GHRPs and their analogs is also partially resistant to inhibition by other substances known to abolish the GHRH-induced GH rise, such as glucose, glucocorticoids, GH which likely stimulates hypothalamic somatostatin release (8, 28, 72, 118, 121), and free fatty acids which likely act directly at the pituitary level by antagonizing depolarization of somatotrope cell membranes (29). On the other hand acipimox, a lipolysis inhibitor, is able to counteract the inhibiting effect of increased circulating free fatty acids on the GH response to GHRP-6 (133). Strengthening the hypothesis that GHRP activity is resistant to inhibitory influences, it has also been shown that the infusion of exogenous somatostatin, at a dose able to abolish the GHRH-induced increase in GH, inhibits but does not abolish the somatotrope responsiveness to Hexarelin (7).

All together, these findings in humans agree with those in animals favoring the hypothesis that the mechanism underlying the GH-releasing activity of GHRPs includes antagonism of somatostatinergic activity at both the pituitary and the hypothalamic levels (65, 84). This may also explain the good intraindividual reproducibility of the GH response to GHRPs (75, 95). To confirm the assumption that the activity of GHRPs depends mainly on a functional hypothalamus, it has been shown that the GH-releasing effect of GHRP-6 and Hexarelin, either alone or in combination with GHRH, is reduced in patients with hypothalamo–pituitary disconnection (116, 140, 143).

In humans, as in animals, there is evidence that GHRPs and GHRH induce homologous but not heterologous desensitization (52, 95, 101, 122, 154, 161, 166). Homologous desensitization to the activity of GHRPs has been shown during GHRP infusion (52, 95, 101), but not after intermittent oral or intranasal daily administration of the peptide for up to 15 days (77, 78). On the other hand, prolonged administration of GHRPs and their analogs by the parenteral, intranasal, or oral route enhances spontaneous GH pulsatility over 24 h and increases IGF-I levels in normal young adults as well as in short children and in elderly subjects (33, 34, 47, 78, 89, 95, 101, 110). These findings indicate that treatment with these compounds is able to augment the activity of GH/IGF-I axis.

ACTH- and PRL-Releasing Effect

As in animals, the activity of GHRPs and nonpeptidyl GHRP mimetics is not fully specific for GH release. Slight but significant and dose-dependent PRL-, ACTH-, and cortisol-releasing effects have been demonstrated (3, 22, 26, 47, 71, 72, 75, 96, 97, 166) (Fig. 4); moreover, the PRL response to Hexarelin was reported to be markedly lower than that recorded after TRH administration (13). On the other hand, a TSH-inhibiting effect has been shown for GHRPs (101, 107).

The mechanisms underlying these effects are still unclear. Both the PRL- and the ACTH/cortisol-releasing activity of Hexarelin are not modified by naloxone (104), cyproheptadine, a serotonin antagonist, or diphenhydramine, a hista-

mine antagonist (12). Thus, the PRL- and ACTH-releasing effects of GHRPs do not appear to depend on mediation by opioids, serotonin, or histamine. These transmitters are known to play an important role in the control of PRL and ACTH secretion (130).

In humans the stimulatory effect of GHRPs on ACTH secretion, which in turn stimulates cortisol release, overlaps with that of naloxone, AVP, and CRH (80, 81, 104). The ACTH-releasing effect of GHRPs is an acute neuroendocrine effect that is lost during prolonged treatment (33, 47) and seems dependent on CNS-mediated mechanisms. Preliminary data suggest that the mechanisms mediating the ACTH-releasing activity of GHRPs in humans are different, at least partially, from those in animals. GHRPs and AVP, but not CRH, have been reported to have synergistical stimulatory effect on ACTH secretion in rats (160).

In man the administration of Hexarelin with CRH, naloxone, or AVP has less than additive effect on ACTH and cortisol secretion (80, 104), despite the well know synergistical effect of CRH and AVP on ACTH secretion (138). These data suggest that in humans the ACTH-releasing activity of GHRPs could be, at least partially, different from CRH- and AVP-mediated mechanisms. On the other hand, the ACTH and cortisol response to Hexarelin is abolished by dexametasone pretreatment, as well as by alprazolam, a benzodiazepine which likely acts via inhibition of hypothalamic CRH release (80). Therefore, the ACTH-releasing activity of GHRPs seems to be sensitive to glucocorticoid feedback and could involve GABAergic-mediated mechanisms.

The hormonal activities of GHRPs are not sex-dependent but show different age-related patterns; in aging, the stimulatory effect of Hexarelin on GH, PRL, and ACTH secretion is reduced, unchanged, and increased, respectively (80). These findings indicate that GHRPs act at different levels and/or on different receptors to induce different endocrine responses.

Pathological Conditions

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

In vitro GHRP-6 and L-692,429 release more GH than GHRH from human pituitary somatotrope adenoma cells (1, 113, 146). Hexarelin was found to stimulate GH release in a GH-secreting rat cell line insensitive to GHRH (83). On the other hand, the mean somatotrope responsiveness to GHRP-6 or Hexarelin, both alone and combined with GHRH, is similar in acromegalics and in normal subjects (4, 5, 41, 87, 142), although there is a dramatic interindividual variability (4). In patients with a somatomammotrope adenoma and acromegaly, the GH and PRL releases induced by Hexarelin were similar to those in normal subjects. However, in patients with idiopathic hyperprolactinemia, there was no change in PRL levels and only a slight GH response (41). A preserved GH-releasing effect of GHRPs was also reported in patients with

functional GH hypersecretory states such as anorexia nervosa (144) and hyperthyroidism (145). The GH response to GHRH has been shown to be exaggerated in anorexia (147) and usually is reduced in hyperthyroidism (145, 164).

GHRPs alone and in combination with GHRH were found to induce a marked GH response in children with idiopathic short stature (16, 17, 107, 108, 115, 116, 136, 163) and even in children and adults with GH deficiency (17, 35, 112, 116, 126, 136, 139, 140, 143, 163). However, in children as well as in adults with pituitary stalk lesions, the GH response to GHRPs alone and combined with GHRH has been found to be greatly impaired (116, 140, 143). The possible usefulness of GHRPs to test the maximal secretory capacity of somatotrope cells in the diagnosis of GH deficiency has been proposed by some authors (107, 108, 112, 116), but others disagree (16, 139).

The possible therapeutic usefulness of GHRPs in children with short stature has been also tested. In open studies, chronic treatment with Hexarelin was found to increase IGF-I levels and growth velocity in children with idiopathic short stature or with GH deficiency (102, 110, 126, 137, 162). During prolonged treatment with MK-0677, a new nonpeptidyl GH secretagogue, a clear IGF-I increase was reported in GH-deficient adults despite small increase in spontaneous GH secretion (35).

A marked GH response to GHRPs, alone and combined with GHRH, was also observed in obese patients in whom reduced GH secretion is known to occur (27, 48, 49, 85, 115). In obesity, inhibition of lipolysis by acipimox enhances the GH response to the combined administration of GHRP-6 and GHRH (50), further pointing to the role of metabolic alterations in the pathogenesis of GH insufficiency in this condition. The GH-releasing effect of GHRPs, both alone and combination with GHRH, was found to be reduced in hypothyroidism (63, R. Valcavi, unpublished data) and nearly absent in Cushing's syndrome (81, 111), 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 (60).

In patients with Cushing's disease the ACTH and cortisol responses to Hexarelin have been reported to be markedly higher than those to CRH, while both Hexarelin and CRH are unable to modify ACTH and cortisol levels in patients with Cushing's syndrome due to adrenal adenoma or ectopic ACTH-secreting tumor (81). This finding suggests that Hexarelin, more so than CRH, may have diagnostic usefulness in differentiating pituitary from ectopic ACTH-dependent Cushing's syndrome.

CONCLUSIONS

About 20 years after their discovery, GHRPs and nonpeptidyl GHRP mimetics are a hot topic in neuroendocrinology, with biologists, clinicians, and pharmaceutical companies actively involved in this field. These agents have been found to be potent GH secretagogues, but they also possess significant

PRL- and ACTH-releasing activity. Although the exact mechanism of action of GHRPs has not been fully established, they probably act on both the pituitary and the hypothalamus and may involve regulatory factors in addition to GHRH and somatostatin. Moreover, the possibility that GHRPs act via an unknown hypothalamic factor (U factor) is still open. The existence of an endogenous GHRP-like ligand is a fascinating possibility and, after the GHRP receptor has been cloned, it should soon be isolated and characterized.

Possible clinical uses for GHRPs and their analogs have been proposed. GHRPs given alone or in combination with GHRH have been suggested as a provocative stimulus to assess the pituitary GH-releasable pool (17, 107, 108, 112, 115, 116, 164, 168). However, since the effect of GHRPs is dependent on age (3, 6, 16, 25, 26, 76, 77, 79, 127), age-related normative values of the GH response to these peptides have to be defined.

Although some promising preliminary results have been reported in short children treated with GHRPs for up to 6 months (110), the efficacy of the GHRPs must be verified in double-blind, placebo controlled studies. Theoretically, GHRPs should be ineffective in GH deficiency due to hypopituitarism. However, GHRP treatment could usefully enhance the activity of the GH/IGF-I axis in GH deficiency due to GH neurosecretory dysfuntion, with the notable exception of patients with hypothalamo–pituitary disconnection (116, 140, 143).

It is still unclear whether it is really useful to restore the activity of the GH/IGF-I axis in elderly people. However, as the pituitary GH-releasable pool is preserved in elderly subjects (74), GH secretagogues are probably the most appropriate approach to restore GH and IGF-I secretion. This may counteract age-related changes in body composition, structure, function, and metabolism (51, 74, 151). It is unlikely that peripheral GH resistance in severe catabolic states (150) may be overridden by increased GHRP-induced GH secretion. On the other hand, GHRPs and their analogs could be useful to increase the activity of the GH/IGF-I axis and to induce anabolism in mild catabolic states or in the phase of recovery from severe catabolism (166). On the basis of recent data reporting the possible usefulness of rhGH in the treatment of dilated cardiomyopathy in the absence of GH deficiency (67), the possible usefulness of GHRPs in this disease also needs to be explored.

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REFERENCES

1. Adams EF, Petersen B, Ting L, Buchfelder M, Fahlbusch R. The growth hormone secretagogue, L-692,429, induces phosphatidylinositol hydrolysis and hormone secretion by human pituitary tumors. *Biochem Biophys Res Commun* 1995; **208:** 555–561.
2. Akman MS, Girard M, O'Brien L, Ho AK, Chik CL. Mechanisms of action of a second generation growth hormone-releasing peptide (Ala-His-D-βNal-Ala-Trp-D-Phe-Lys-NH2) in rat anterior pituitary cells. *Endocrinology* 1993; **132:** 1286–1291.
3. Aloi JA, Gertz BJ, Hartman ML, Huhn WC, Pezzoli SS, Wittreich M, Krupa DA, Thorner MO. Neuroendocrine responses to a novel growth hormone secretagogue, L-692,429, in healthy older subjects. *J Clin Endocrinol Metab* 1994; **79:** 943–949.
4. Alster DK, Bowers CY, Jaffe CA, Ho PJ, Barkan AL. The growth hormone (GH) response to GH-releasing peptide (His-D-Trp-Ala-Trp-D-Phe-Lys-NH2), GH-releasing hormone, and thyrotropin-releasing hormone in acromegaly. *J Clin Endocrinol Metab* 1993; **77:** 842–845.
5. Arosio M, Biella O, Casati G, Guglielmino L, Palmieri E, Faglia G. Effects of Hexarelin (EP23905-MF6003) on circulating GH levels in acromegalic patients. (Highlights in *Mol Clin Endocrinol*). *Front Endocrinol* 1994; **9:** 151–154.
6. Arvat E, Gianotti L, Grottoli S, Imbimbo BP, Lenaerts V, Deghenghi R, Camanni F, Ghigo E. Arginine and growth hormone-releasing hormone restore the blunted growth hormone-releasing activity of hexarelin in elderly subjects. *J Clin Endocrinol Metab* 1994; **79:** 1440–1443.
7. Arvat E, Gianotti L, Di Vito L, Imbimbo BP, Lenaerts V, Deghenghi R, Camanni F, Ghigo E. Modulation of growth hormone-releasing activity of hexarelin in man. *Neuroendocrinology* 1995; **61:** 51–56.
8. Arvat E, Di Vito L, Gianotti L, Ramunni J, Boghen MF, Deghenghi R, Camanni F, Ghigo E. Mechanisms underlying the negative growth hormone (GH) autofeedback on the GH-releasing effect of Hexarelin in man. *Metabolism* 1997; **46:** 83–88.
9. Arvat E, Ceda GP, Ramunni J, DiVito L, Gianotti L, Boghen MF, Deghenghi R, Ghigo E. Supramaximal doses of GHRH or Hexarelin, a synthetic hexapeptide, enhance the low somatotrope responsiveness in aging. *J Endocrinol Invest* 1996; **19**(Suppl. 5):3.
10. Arvat E, Gianotti L, Ramunni J, Di Vito L, Deghenghi R, Camanni F, Ghigo E. Influence of beta-adrenergic agonists and antagonists on the GH-releasing effect of Hexarelin in man. *J Endocrinol Invest* 1996; **19:** 25–29.
11. Arvat E, Gianotti L, Broglio F, Maccagno B, Bertagna A, Deghenghi R, Camanni F, Ghigo E. Oestrogen replacement does not restore the reduced GH-releasing activity of hexarelin, a synthetic hexapeptide, in postmenopausal women. *Eur J Endocrinol* 1997; **136:** 483–487.
12. Arvat E, Maccagno B, Ramunni J, Gianotti L, Di Vito L, Deghenghi R, Camanni F, Ghigo E. Effects of histaminergic antagonists on the GH-releasing activity of GHRH or hexarelin, a synthetic hexapeptide, in man. *J Endocrinol Invest* 1997; **20:** 122–127.
13. Arvat E, Di Vito L, Maccagno B, Broglio F, Boghen MF, Deghenghi R, Camanni F, Ghigo E. Effects of GHRP-2 and Hexarelin, two synthetic GH-releasing peptides, on GH, prolactin, ACTH and cortisol level in man. Comparison with the effects of GHRH, TRH, and hCRH. *Peptides*, 1997; **18:** 885–891.
14. Badger TM, Millard WY, McCormik GF, Bowers CY, Martin JB. The effects of growth hormone (GH)-releasing peptides on GH secretion in perifused pituitary cells of adult male rats. *Endocrinology* 1984; **115:** 1432–1438.
15. Blake AD, Smith RG. Desensitization studies using perifused rat pituitary cells show that growth hormone-releasing hormone and His-D-Trp-D-Phe-Lys-NH2 stimulate GH release through different receptor sites. *J Endocrinol* 1991; **129:** 11–19.
16. Bellone J, Aimaretti G, Bartolotta E, Benso L, Imbimbo BP, Lenhaerts V, Deghenghi R, Camanni F, Ghigo E. Growth hormone-releasing activity of Hexarelin, a new synthetic hexapeptide, before and during puberty. *J Clin Endocrinol Metab* 1995; **80:** 1090–1094.

17. Bellone J, Ghizzoni L, Aimaretti G, Volta C, Boghen MF, Bernasconi S, Ghigo E. Growth hormone-releasing effect of oral growth hormone-releasing peptide 6 (GHRP-6) administration in children with short stature. *Eur J Endocrinol* 1995; **133:** 425–429.

18. Bercu BB, Weideman CA, Walker RF. Sex differences in growth hormone (GH) secretion by rats administered GH-releasing hexapeptide. *Endocrinology* 1991; **129:** 2592–2598.

19. Bercu BB, Yang SW, Masuda R, Walker RF. Role of selected endogenous peptides in growth hormone-releasing hexapeptide activity: Analysis of growth hormone-releasing hormone, thyroid hormone-releasing hormone, and gonadotropin-releasing hormone. *Endocrinology* 1992; **130:** 2579–2586.

20. Bitar KG, Bowers CY, Coy DH. Effect of substance P/bombesin antagonists on the release of GH by GHRP and GHRH. *Biochem Biophys Res Commun* 1991; **5:** 156–161.

21. Bowers CY, Momany FA, Reynolds GA, Hong A. On the in vitro and in vivo activity of a new synthetic hexapeptide that acts on the pituitary to specifically release growth hormone. *Endocrinology* 1984; **114:** 1537–1545.

22. Bowers CY, Reynolds GA, Durham D, Barrera CM, Pezzoli SS, Thorner MO. Growth hormone (GH)-releasing peptide stimulates GH release in normal men and acts synergistically with GH-releasing hormone. *J Clin Endocrinol Metab* 1990; **70:** 975–982.

23. Bowers CY, Sartor AO, Reynolds GA, Badger TM. On the action of the growth hormone-releasing hexapeptide, GHRP. *Endocrinology* 1991; **128:** 2027–2035.

24. Bowers CY, Alster DK, Frentz JM. The growth hormone-releasing activity of a synthetic hexapeptide in normal men and short statured children after oral administration. *J Clin Endocrinol Metab* 1992; **74:** 292–298.

25. Bowers CY, Veeraragavan K, Sethumadhavan K. Atypical growth hormone releasing peptides. In: Bercu BB, Walker RF, Eds. *Growth Hormone II, Basic and Clinical Aspects.* Springer-Verlag: New York, 1993: 203–222.

26. Bowers CY. GH releasing peptides: Structure and kinetics. *J Pediatr Endocrinol* 1993; **6:** 21–31.

27. Bowers CY. Novel GH-releasing peptides. In: Melmed S Ed. *Molecular and Clinical Advances in Pituitary Disorders.* Los Angeles: Endocr Res Educ, INC, 1993: 153.

28. Cappa M, Setzu S, Bernardini S, Carta D, Federici G, Grossi A, Loche S. Exogenous growth hormone administration does not inhibit the growth hormone response to Hexarelin in normal men. *J Endocrinol Invest* 1995; **18:** 762–766.

29. Casanueva FF. Physiology of growth hormone secretion and action. In: Melmed S, Ed. *Endocrinology and Metabolism Clinics of North America.* Philadelphia, Saunders, 1992: 483–517.

30. Cella S, Locatelli V, Poratelli M, De Gennaro Colonna V, Imbimbo BP, Deghenghi R, Muller EE. Hexarelin, a potent GHRP analogue: Interaction with GHRH and clonidine in young and aged dogs. *Peptides* 1995; **16:** 81–86.

31. Chang CH, Rickes EL, Marsilio F, McGuire L, Cosgrove S, Taylor J, Chen H, Feighner S, Clark JN, De Vita R, Schoen W, Wyvratt M, Fisher M, Smith RG, Hickey GJ. Activity of a novel non peptidyl GH secretagogue, L-700,653, in swine. *Endocrinology* 1995; **136:** 1065–1071.

32. Chang CH, Faidley T, Rickes L, McGuire LA, Nicolich S, Murphy T, Frazier E, Chen H, Smith RG, Hickey J. Evidence that L-765,867 inhibits GH secretion when infused intravenously over a 12-hour period in swine. 10th International Congress of Endocrinology, San Francisco 1996: 486.

33. Chapman IM, Bach MA, Van Cauter E, Farmer M, Krupa D, Taylor AM, Schilling LM, Cole KY, Skiles EH, Pezzoli SS, Hartman ML, Veldhuis JD, Gormley GJ, Thorner MO. Stimulation of the growth hormone (GH)-insulin-like growth factor I axis by daily oral administration of a GH secretagogue (MK-677) in healthy elderly subjects. *J Clin Endocrinol Metab* 1996; **81:** 4249–4257.

34. Chapman IM, Hartman ML, Pezzoli SS, Thorner MO. Enhancement of pulsatile growth hormone secretory by continous infusion of a growth hormone-releasing peptide mimetic, L-692,429, in older adults—A clinical research center study. *J Clin Endocrinol Metab* 1996; **81:** 2874–2880.

35. Chapman IM, Pescovitz OH, Treep T, Murphy G, Cerchio K, Krupa D, Gertz B, Polvino W, Skiles EH, Pezzoli SS, Thorner MO. Oral administration of the GHRP-mimetic MK-0677 stimulates the GH/IGF-I axis in GH deficient adults. 10th International Congress of Endocrinology, San Francisco 1996: 769.

36. Chatelain P, Claris O, Lapillonne A, Dimayo M, Salle B. Fetal and neonatal somatotroph function. *Front Paediatr Neuroendocrinol* 1994; **20:** 127–130.

37. Cheng K, Chan WW, Barreto A, Convey EM, Smith RG. The synergistic effects of His-D-Trp-Ala-Trp-D-Phe-Lys-NH2 on growth hormone (GH)-releasing factor-stimulated GH release and intracellular adenosine 3′,5′-monophosphate accumulation in rat primary cell culture. *Endocrinology* 1989; **124:** 2791–2798.

38. Cheng K, Chan WW, Butler B, Wei L, Smith RG. A novel non-peptidyl growth hormone secretagogue. *Horm Res* 1993; **49:** 109–115.

39. Cheng K, Chan WW, Butler B, Wei L, Schoen WR, Wyratt MJ, Fisher MH, Smith RG. Stimulation of growth hormone release from rat primary pituitary cells by L-692,429, a novel non-peptidyl GH secretagogue. *Endocrinology* 1993; **132:** 2729–2731.

40. Chihara K, Kaji H, Hayashi S, Yagi H, Takeshima Y, Mitani M, Ohashi SI, Abe H. Growth hormone releasing hexapeptide: Basic research and clinical application. In: Bercu BB, Walker RF, Eds. *Growth Hormone II, Basic and Clinical Aspects.* Springer-Verlag, New York, 1993: 203–222.

41. Ciccarelli E, Grottoli S, Razzore P, Gianotti L, Arvat E, Deghenghi R, Camanni F, Ghigo E. Hexarelin, a synthetic growth hormone releasing peptide, stimulates prolactin secretion in acromegalic but not in hyperprolactinemia patients. *Clin Endocrinol* 1996; **44:** 67–71.

42. Clark RG, Carlsson MS, Trojnar J, Robinson ICAF. The effects of a growth hormone-releasing peptide and growth hormone-releasing factor in conscious and anaesthetized rats. *J Neuroendocrinol* 1989; **1:** 249–255.

43. Clark RG, Mortenesen DL, Won WB, Ma YH, Stanley MS, Somers TC, Fairhall KM, Thoams GB, Robinson ICAF. Novel GHRP analogs acutely activate the HPA axis and are diabetogenic in ZDF rats. 10th International Congress of Endocrinology, San Francisco 1996; 282.

44. Codd EE, Yellin T, Walker RF. Binding of growth hormone-releasing hormone and enkephalin-derived growth hormone-releasing peptides to mu and delta opioid receptors in forbrain of rat. *Neuropharmacology* 1988; **27:** 1019–1025.

45. Codd EE, Shu AYL, Walker RF. Binding of a growth hormone releasing hexapaptide to specific hypothalamic and pituitary binding sites. *Neuropharmacology* 1989; **28:** 1139–1144.

46. Conley LK, Teik JA, Deghenghi R, Imbimbo BP, Giustina A, Locatelli V, Wehrenberg WB. Mechanism of action of Hexarelin and GHRP-6: Analysis of the involvement of GHRH and somatostatin in the rat. *Neuroendocrinology* 1995; **61:** 44–50.

47. Copinschi G, van Onderbergen A, L'hermite-Baleriaux M, Mendel CM, Caufriez A, Leproult R, Bolognese JA, De Smet M, Thorner MO, van Cauter E. Effects of 7-day treatment with a novel, orally active, growth hormone (GH) secretagogue, MK-677, on 24-hour GH profiles, insulin-like growth factor I, and adrenocortical function in normal young men. *J Clin Endocrinol Metab* 1996; **81:** 2776–2782.

48. Cordido F, Penalva A, Dieguez C, Casanueva FF. Massive growth hormone (GH) discharge in obese subjects after the combined administration of GH-releasing hormone and GHRP-6: Evidence for a marked somatotroph secretory capability in obesity. *J Clin Endocrinol Metab* 1993; **76:** 819–823.

49. Cordido F, Penalva A, Peino R, Casanueva FF, Dieguez C. Effect of combined administration of growth hormone (GH)-releasing hormone, GH-releasing peptide-6 and pyridostigmine in normal and obese subjects. *Metabolism* 1995; **44:** 745–748.

50. Cordido F, Peino R, Penalva A, Alvarez CV, Casanueva FF, Dieguez C. Impaired growth hormone secretion in obese subjects is partially reversed by acipimox-mediated plasma free fatty acid depression. *J Clin Endocrinol Metab* 1996; **81:** 914–918.

51. Corpas E, Harman SM, Blackman G. Human growth hormone and human aging. *Endocr Rev* 1993; **14:** 20–39.

52. DeBell WK, Pezzoli SS, Thorner MO. Growth hormone (GH) secretion during continuous infusion of GH-releasing peptide: Partial response attenuation. *J Clin Endocrinol Metab* 1991; **72:** 1312–1316.

53. Deghenghi R. Growth hormone releasing peptides. In: Bercu B, Walker R, Eds. *Growth Hormone Secretagogues*. Serono Symposia, New York, 1993: 85–102.

54. Deghenghi R, Cananzi M, Torsello A, Battisti C, Muller EE, Locatelli V. GH-releasing activity of Hexarelin, a new growth hormone releasing peptide, in infant and adult rats. *Life Sci* 1994; **54:** 1321–1328.

55. Deghenghi R, Boutignon F, Luoni M, Grilli R, Guidi M, Locatelli V. Small peptides as potent releasers of growth hormone. *J Ped Endocrinol Metab* 1995; **8:** 311–313.

56. Deghenghi R, Boutignon F, Luoni F, Grilli R, Guidi M, Locatelli V. Characterization of the activity of new growth hormone secretagogues in the infant rat. 10th International Congress of Endocrinology, San Francisco 1996: 280.

57. DeVita RJ, Wyvratt MJ. Benzolactam growth hormone secretagogues. *Drugs Future* 1996; **21:** 273–281.

58. Dickson SL, Leng G, Dyball REJ, Smith RG. Central actions of peptide and non-peptide growth hormone secretagogues in the rat. *Neuroendocrinology* 1995; **61:** 36–43.

59. Dickson SL, Doutrelant-Viltart O, Leng G. 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-6. *J Endocrinol* 1995; **146:** 519–526.

60. Dieguez C, Mallo F, Senaris R, Pineda J, Martul P, Leal-Cerro A, Pombo M, Casanueva FF. Role of glucocorticoids in the neuroregulation of growth hormone secretion. *J Pediatr Endocrinol Metab* 1996; **9:** 255–260.

61. Doscher ME, Baker PK, Kraft LA, Ricks CA. Effect of a synthetic growth hormone releasing hexapeptide (BI 679) and growth hormone releasing factor (GRF) on serum growth hormone levels in barrows. *J Anim Sci* 1984; **59:** 218.

62. Dubreuil P, Petitclerc D, Pelletier G, Gaudeau P, Farmer C, Mowles TF, Brezean P. Effect of dose and frequency of administration of a potent analog of human growth hormone-releasing factor on hormone secretion in pigs. *J Anim Sci* 1990; **68:** 1254–1268.

63. Edwards CA, Dieguez C, Scanlon MF. Effects of hypothyroidism, tri-iodothyronine and glucocorticoids on growth hormone responses to growth hormone-releasing hormone and His-D-Trp-Ala-Trp-D-Phe-Lys-NH2. *J Endocrinol* 1989; **121:** 31–36.

64. Elias KA, Ingle GS, Burnier JP, Hammonds RG, McDowell RS, Rawson TE, Somers TC, Stanley MS, Cronin MJ. In vitro characterization of four novel classes of growth hormone-releasing peptide. *Endocrinology* 1995; **136:** 5694–5699.

65. Fairhall KM, Mynett A, Robinson ICAF. Central effects of growth hormone-releasing hexapeptide (GHRP-6) on growth hormone release are inhibited by central somatostatin action. *J Endocrinol* 1995; **144:** 555–560.

66. Fairhall KM, Mynett A, Smith RG, Robinson ICAF. Consistent GH responses to repeated injections of GH-releasing hexapeptide (GHRP-6) and the non-peptide GH secretagogue, L-692,585. *J Endocrinol* 1995; **145:** 417–426.

67. Fazio S, Sabatini D, Capaldo B, Vigorito C, Giordamo A, Guida R, Pardo F, Biondi B, Saccà L. A preliminary study of growth hormone in the treatment of dilated cardiomyopathy. *N Engl J Med* 1996; **334:** 100–103.

68. Fletcher TP, Thomas GB, Willoughby JO, Clarke IJ. Constitutive growth hormone secretion in sheep after hypothalamopituitary disconnection and the direct in vivo pituitary effect of growth hormone releasing peptide 6. *Neuroendocrinology* 1994; **60:** 76–86.

69. Frieboes RM, Murck H, Maier P, Schier T, Holsboer F, Steiger A. Growth hormone-releasing peptide-6 stimulates sleep, growth hormone, ACTH and cortisol release in normal man. *Neuroendocrinology* 1995; **61:** 584–589.

70. Gelato MC, Pescovitz DH, Cassorla F, Loriaux DL, Merriam GR. Dose-response relationships for the effects of GH-releasing factor 1-44 NH2 in young adult men and women. *J Clin Endocrinol Metab* 1984; **59:** 197–203.

71. Gertz BJ, Barrett JS, Eisenhandler R, Krupa DA, Wittreich JM, Seibold JR, Schneider SH. Growth hormone response in man to L-692,429, a novel non peptide mimic of growth hormone-releasing peptide-6. *J Clin Endocrinol Metab* 1993; **77:** 1393–1397.

72. Gertz BJ, Sciberras DG, Yogendran L, Christie K, Bador K, Krupa D, Wittreich JM, James I. L-692,429, a non peptide growth hormone (GH) secretagogue reverses glucocorticoid suppression of GH secretion. *J Clin Endocrinol Metab* 1994; **79:** 745–749.

73. Ghigo E. Neurotransmitter control of growth hormone secretion: In: de la Cruz LF Ed. *Growth Hormone and Somatic Growth.* Excerpta Medica; Amsterdam 1992; 103–136.

74. Ghigo E, Arvat E, Goffi S, Bellone J, Nicolosi M, Procopio M, Maccario M, Camanni F. Neural control of growth hormone secretion in aged humans. In: Muller EE, Cocchi D, Locatelli V, Eds. *Growth Hormone and Somatomedins during Lifespan.* Springer-Verlag, Berlin 1993: 275–287.

75. Ghigo E, Arvat E, Gianotti L, Imbimbo BP, Lenaerts V, Deghenghi R, Camanni F. Growth hormone-releasing activity of Hexarelin, a new synthetic hexapeptide, after intravenous, subcutaneous, intranasal and oral administration in man. *J Clin Endocrinol Metab* 1994; **78:** 693–698.

76. Ghigo E, Arvat E, Rizzi G, Bellone J, Nicolosi, Boffano GM, Boghen MF, Camanni F. Arginine enhances the growth hormone (GH)-releasing activity of a synthetic hexapeptide (GHRP-6) in elderly but not in young subjects after oral administration. *J Endocrinol Invest* 1994; **17:** 157–162.

77. Ghigo E, Arvat E, Rizzi G, Goffi S, Mucci M, Boghen MF, Camanni F. Growth hormone-releasing activity of growth hormone-releasing peptide-6 is maintained after short-term oral pretreatment with the hexapeptide in normal aging. *Eur J Endocrinol* 1994; **131:** 499–503.

78. Ghigo E, Arvat E, Gianotti L, Grottoli S, Rizzi G, Ceda GP, Boghen MF, Deghenghi R, Camanni F. Short term administration of intranasal or oral hexarelin, a synthetic hexapeptide, does not desensitize the GH responsiveness in human aging. *Eur J Endocrinol* 1996; **135:** 407–412.

79. Ghigo E, Arvat E, Gianotti L, Grottoli S, Rizzi G, Ceda GP, Deghenghi R, Camanni F. Aging and GH-releasing peptides. In: Bercu B, Walker R, Eds. Growth Hormone Secretagogues. Springer-Verlag, New York: 1996: 415–431.

80. Ghigo E. Effects of GHRPs during lifespan in humans. 10th International Congress of Endocrinology, San Francisco 1996: 26.

81. Ghigo E, Arvat E, Ramunni J, Colao A, Gianotti L, Deghenghi R, Lombardi G, Camanni F. Adrenocorticotropin- and cortisol-releasing effect of hexarelin, a synthetic growth hormone-releasing peptide, in normal subjects and patients with Cushing's syndrome. *J Clin Endocrinol Metab* 1997; **89:** 2439–2444.

82. Giustina A, Bussi AR, Deghenghi R, Imbimbo BP, Licini M, Poiesi C, Wehrenberg WB. Comparison of the effects of growth hormone-releasing hormone and hexarelin, a novel growth hormone-releasing peptide-6 analog, on growth hormone secretion in humans with or without glucocorticoid excess. *J Endocrinol* 1995; **146:** 227–232.

83. Giustina A, Bonfani C, Licini M, Ragni G, Stefana B. Hexarelin, a novel GHRP-6 analog, stimulates growth hormone (GH) release in a GH-secreting rat cell line (GH_1) insensitive to GH-releasing hormone. 10th International Congress of Endocrinology, San Francisco 1996: 288.

84. Goth MI, Lyons CE, Canny BY, Thorner MO. Pituitary adenylate cyclase activating polypeptide, growth hormone (GH)-releasing peptide and GH-releasing hormone stimulate GH release through distinct pituitary receptors. *Endocrinology* 1992; **130:** 939–944.

85. Grottoli S, Maccario M, Procopio M, Oleandri S, Razzore P, Taliano M, Camanni F, Ghigo E. Selective refractoriness of somatotrope secretion to the inhibitory effect of hyperglycemia in obesity. *Eur J Endocrinol* 1996; **135:** 678–682.

86. Guillame V, Magnam E, Cataldi M, Dutour A, Sauze N, Renard M, Rezafindraibe H, Conte Devol B, Lenaertz V, Oliver C. Growth hormone (GH)-releasing hormone secretion is stimulated by a new GH-releasing hexapeptide in sheep. *Endocrinology* 1994; **135:** 1073–1076.

87. Hanew K, Utsumi A, Sugawara A, Shimizu Y, Abe K. Enhanced GH responses to combined administration of GHRP and GHRH in patients with acromegaly. *J Clin Endocrinol Metab* 1994; **78:** 509–512.

88. Hartman ML, Farello G, Pezzoli SS, Thorner MO. Oral administration of growth hormone (GH)-releasing peptide stimulates GH secretion in normal men. *J Clin Endocrinol Metab* 1992; **74:** 1378–1384.

89. Hayashi S, Okimura Y, Yagi H, Uchiyama T, Takeshima S, Shakatsui S. Intranasal administration of His-D-Trp-Ala-Trp-D-Phe-Lys-NH2 (growth hormone releasing peptide) increased plasma growth hormone and insulin-like growth factor-I levels in normal men. *Endocrinol Jpn* 1991; **38:** 15–21.

90. Herrington J, Hille B. Growth hormone-releasing hexapeptide elevates intracellular calcium in rat somatotropes by two mechanisms. *Endocrinology* 1994; **135:** 1100–1108.

91. Hickey GJ, Jacks T, Judith F, Taylor J, Schoen WR, Krupa D, Cunningham P, Clark J, Smith RG. Efficacy and specificity of L-692,429, a novel nonpeptidyl growth hormone secretagogue, in beagles. *Endocrinology* 1994; **134:** 695–701.

92. Hickey GJ, Drisko J, Faidley T, Chang C, Anderson L, Nicolich S, McGuire L, Rickes E, Krupe D, Feeney W, Friscino B, Cunningham P, Frazier E, Chen H, Leroque P, Smith RG. Mediation by the central nervous system is critical to the in vivo activity of the GH secretagogue L-692,585. *J Endocrinol* 1996; **148:** 371–380.

93. Hickey GJ, Jacks T, Schleim K, Frazier E, Chen H, Krupa D, Feeney W, Narpunet R, Patchett A, Smith RG. Repeated administration of the GH secretagogue MK-0677 increases and maintains elevated IGF-1 levels. 10th International Congress of Endocrinology, San Francisco 1996: 285.

94. Howard AD, Feighner SD, Cully DF, Arena JP, Liberator PA, Rosenblum CI, Uamelin M, Ureniuk DI, Palyhe OC, Anderson J, Paress PS, Diaz C, Chou N, Liu KK, McKee KK, Pong SS, Cheung LY, Elbrecht A, Dashkevicz M, Keavehs R, Rishy M, Siringjhsinghji DJS, Dean DC, Melillo DG, Patchett AA, Nergund R, Srillin PR. A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* 1996; **273:** 974–977.

95. Huhn WC, Hartman ML, Pezzoli SS, Thorner MO. Twenty-four-hour growth hormone (GH)-releasing peptide (GHRP) infusion enhances pulsatile GH secretion and specifically attenuates the response to a subsequent GHRP bolus. *J Clin Endocrinol Metab* 1993; **76:** 1202–1208.

96. Ilson BE, Jorkasky DK, Curnow RT, Stote RM. Effect of a new synthetic hexapeptide to selectively stimulate growth hormone release in healthy human subjects. *J Clin Endocrinol Metab* 1989; **69:** 212–214.

97. Imbimbo BP, Mant T, Edward M, Amin D, Froud A, Lenaerts U, Boutignon F, Deghenghi R. Growth hormone releasing activity of hexarelin in humans: A dose–response study. *Eur J Clin Pharmacol* 1994; **46:** 421–425.

98. Ishii S, Okada K, Suzuki N, Sugihara H, Minami S, Shibasaki T, Wakabayashi I. Effect of growth hormone-releasing peptide (KP-102) on food intake in rats. 10th International Congress of Endocrinology, San Francisco 1996: 282.

99. Jacks T, Hickey G, Judith F, Taylor J, Chen H, Krupa D, Feeney W, Schoen W, OK Fisher M, Wyratt M, Smith R. Effects of acute and repeated intravenous administration of L-692,585, a novel non-peptidyl growth hormone secretagogue, on plasma growth hormone, IGF-I, ACTH, cortisol, prolactin, insulin, and thyroxine levels in beagles. *J Endocrinol* 1994; **143:** 399–406.

100. Jacks T, Smith R, Judith F, Schleim K, Frazier E, Chen H, Krupa D, Hora D, Nargund R, Patchett A, Hickey G. MK-0677, a potent novel, orally-active growth hormone (GH) secretagogue: GH, insulin-like growth factor I, and other hormonal responses in beagles. *Endocrinology* 1996; **137:** 5284–5289.

101. Jaffe CA, Ho PJ, Demott-Friberg R, Bowers CY, Barkan AL. Effects of a prolonged growth hormone (GH)-releasing peptide infusion on pulsatile GH secretion in normal men. *J Clin Endocrinol Metab* 1993; **77:** 1641–1647.

102. Klinger B, Silbergeld A, Deghenghi R, Frenkel J, Laron Z. Desensitization from long-term intranasal treatment with Hexarelin does not interfere with the biological effects of this growth hormone-releasing peptide in short children. *Eur J Endocrinol* 1996; **134:** 716–719.

103. Korbonits M, Grossman AB. Growth hormone-releasing peptide and its analogues. Novel stimuli to growth hormone release. *Trends Endocrinol Metab* 1995; **6:** 43–49.

104. Korbonits M, Trainer PJ, Besser GM. The effect of an opiate antagonist on the hormonal changes induced by hexarelin. *Clin Endocrinol* 1995; **43:** 365–371.

105. Korbonits M, Little JA, Trainer PJ, Besser GM, Grossman AB. Analogues of growth hormone-releasing peptide (GHRP) do not affect the release of growth hormone-releasing hormone (GHRH) or somatostatin from the rat hypothalamus. *J Endocrinol* 1996; **148** (Suppl): 123.

106. Kraft LA, Baker PK, Doscher ME, Ricks CA. Effect of a synthetic growth hormone releasing hexapeptide on serum growth hormone levels in steers. *J Anim Sci* 1984; **59:** 218.

107. Laron Z, Bowers CY, Hirsch D, Almonte AS, Pelz N, Keret R, Gil-Ad I. Growth hormone-releasing activity of growth hormone-releasing peptide-1 (a synthetic heptapeptide) in children and adolescents. *Acta Endocrinol* 1993; **129:** 424–429.

108. Laron Z Frenkel J, Gill-Ad, Klinger B, Imbim E, Wuthrich P, Boutignon F, Lengerts V, Deghenghi R. Growth hormone releasing activity by intranasal administration of a synthetic hexapeptide (Hexarelin). *Clin Endocrinol* 1994; **41:** 539– 541.

109. Laron Z. Growth hormone secretagogues. *Drugs* 1995; **50:** 595–601.

110. Laron Z, Frenkel J, Deghenghi R, Anin S, Klinger B, Silbergeld A. Intranasal administration of the GHRP Hexarelin accelerates growth in short children. *Clin Endocrinol* 1995; **43:** 631–635.

111. Leal Cerro A, Pumar A, Garcia-Garcia E, Dieguez C, Casanueva FF. Inhibition of growth hormone release after the combined administration of GHRH and GHRP-6 in patients with Cushing's syndrome. *Clin Endocrinol* 1994; **41:** 649–654.

112. Leal-Cerro A, Garcia E, Astorga R, Casanueva FF, Dieguez C. Growth hormone (GH) responses to the combined administration of GH-releasing peptide 6 in adults with GH deficiency. *Eur J Endocrinol* 1995; **132:** 712–715.

113. Lei T, Buchfelder M, Fahlbusch R, Adams EF. Growth hormone releasing peptide (GHRP-6) stimulates phosphatidylinositol (PI) turnover in human pituitary somatotroph cells. *J Mol Endocrinol* 1995; **14:** 135–138.

114. Locatelli V, Grilli R, Torsello A, Cella SG, Wehrenberg WB, Muller EE. Growth hormone-releasing hexapeptide is a potent stimulator of growth hormone gene expression and release in the growth hormone-releasing hormone-deprived infant rat. *Ped Res* 1994; **36**(2): 169–174.

115. Loche S, Cambiaso P, Carta D, Setzu S, Imbimbo BP, Borrelli P, Pintor C, Cappa M. The growth hormone-releasing activity of Hexarelin, a new synthetic hexapeptide, in short normal and obese children and in hypopituitary subjects. *J Clin Endocrinol Metab* 1995; **80:** 674–678.

116. Loche S, Colao A, Cappa M, Bellone J, Aimaretti G, Farello G, Faedda A, Lombardi G, Deghenghi R, Ghigo E. The growth hormone response to Hexarelin in children: Reproducibility and effect of sex steroids. *J Clin Endocrinol Metab* 1997; **82:** 861–864.

117. Locke W, Kirgis HD, Bowers CY, Abdo AA. Intracerebroventricular growth-hormone-releasing peptide-6 stimulates eating without affecting plasma growth hormone responses in rats. *Life Sci* 1995; **56:** 1347–1352.

118. Maccario M, Arvat E, Procopio M, Gianotti L, Grottoli S, Imbimbo BP, Deghenghi R, Camanni F, Ghigo E. Metabolic modulation of the growth-hormone-releasing activity of hexarelin in man. *Metabolism* 1995; **44**(1): 134–138.

119. Mallo F, Alvarez CV, Benitez L, Burguera B, Coya R, Casanueva FF, Dieguez C. Regulation of His-D-Trp-Ala-Trp-D-Phe-Lys-NH2 (GHRP6)-induced GH secretion in the rat. *Neuroendocrinology* 1993; **57:** 247–256.

120. Malozowski S, Hao EN, Ren SG, Marin G, Liu L, Southers JL, Merriam GR. Growth hormone (GH) responses to the hexapeptide GH-releasing peptide and GH-releasing hormone (GHRH) in the cynomologus macaque: Evidence for non-GHRH-mediated responses. *J Clin Endocrinol Metab* 1991; **73:** 314–317.

121. Massoud AF, Hindmrsh PC, Brook CG. Hexarelin induced growth hormone release is influenced by exogenous growth hormone. *Clin Endocrinol* 1995; **43:** 617–621.

122. Massoud AF, Hindmarsh PC, Matthews DR, Brook CGD. The effect of repeated administration of hexarelin, a growth hormone releasing peptide, and growth hormone releasing hormone on growth hormone responsivity. *Clin Endocrinol* 1996; **44:** 555–562.

123. Mazza E, Ghigo E, Goffi S, Procopio M, Imperiale E, Arvat E, Bellone J, Boghen MF, Muller EE, Camanni F. Effect of the potentiation of cholinergic activity on the variability in individual GH response to GHRH. *J Endocrinol Invest* 1989; **12:** 795–798.

124. McCormick GF, Millard WJ, Badger TN, Bowers CY, Martin JB. Dose–response characteristics of various peptides with growth hormone-releasing activity in the unanesthetized male rat. *Endocrinology* 1985; **117:** 97–105.

125. McDowell RS, Elias KA, Stanley MS, Burdick DJ, Burnier JP, Chan KS, Fairbrother WJ, Hemmonets RG, Mortensen DL, Rawson TE, Wvn WB, Clark RG, Somers TC. Growth hormone secretagogues: Characterization, efficacy, and minimal bioactive conformation. *Biochemistry* 1995; **92:** 11165–11169.

126. Mericq V, Cassorla F, Garcia H, Avila A, Bowers CY, Merriam GR. Growth hormone (GH) responses to GH-releasing peptide and to GH-releasing hormone in GH-deficient children. *J Clin Endocrinol Metab* 1995; **80:** 1681–1684.

127. Micic D, Popovic V, Kendereski A, Macul D, Casanueva FF, Dieguez C, Laron Z. Growth hormone secretion after the administration of GHRP-6 or GHRH combined with GHRP-6 does not decline in late adulthood. *Clin Endocrinol* 1995; **42:** 191–194.

128. Momany FA, Bowers CY, Reynolds GA, Hong A, Newlander K. Conformational energy studies and in vitro and in vivo activity data on active GH releasing peptides. *Endocrinology* 1984; **114:** 1531–1536.

129. Muccioli G, Ghe' C, Ghigo MC, Arvat E, Papotti M, Boghen MF, Deghenghi R, Ong H, Ghigo E. Growth hormone-releasing peptide receptors in human brain and pituitary gland. *Fundam Clin Pharmacol* 1996; **10**(Suppl): 205.

130. Muller EE, Nistico' G, Eds. *Brain Messenger and the Pituitary.* San Diego: Academic Press, 1989.

131. Muruais J, Penalva A, Dieguez C, Casanueva FF. Influence of endogenous cholinergic tone and alpha-adrenergic pathways on growth hormone responses to His-D-Trp-Ala-Trp-D-Phe-Lys-NH2 in the dog. *J Endocrinol* 1993; **138:** 211–218.

132. Patchett AA, Nargunt RP, Tata JR, Chen MH, Barekat KJ, Johnston DBR, Cheng K, Chan WWS, Butler B, Hickey G, Jacks T, Schkeim K, Pong SS, Cheung LYP, Chen HY, Frezier E, Leung KH, Chiu SH, Smith RG. Design and biological activities of L-163,191 (MK-0677): A

potent orally active growth hormone secretagogue. *Proc Natl Acad Sci USA* 1995; **92:** 7001–7005.

133. Peino R, Cordido F, Penalva A, Alvarez V, Dieguez C, Casanueva FF. Acipimox-mediated plasma free fatty acid depression per se stimulates growth hormone (GH) secretion in normal subjects and potentates the response to other GH-releasing stimuli. *J Clin Endocrinol Metab* 1996; **81:** 909–913.
134. Penalva A, Carballo A, Pombo M, Casanueva FF, Dieguez C. Effect of growth hormone (GH)-releasing hormone (GHRH), atropine, pyridostigmine, or hypoglycemia on GHRP-6-induced GH secretion in man. *J Clin Endocrinol Metab* 1993; **76:** 168–171.
135. Penalva A, Pombo M, Carballo A, Barreiro J, Casanueva FF, Dieguez C. Influence of sex, age and adrenergic pathways on the growth hormone response to GHRP-6. *Clin Endocrinol (Oxford)* 1993; **38:** 87–91.
136. Pihoker C, Middleton R, Reynolds GA, Bowers CY, Badger TM. Diagnostic studies with intravenous and intranasal growth hormone-releasing peptide-2 in children of short stature. *J Clin Endocrinol Metab* 1995; **80:** 2987–2992.
137. Pihoker C, Badger TM, Reynolds GA, Bowers CY. Intranasal growth hormone (GH) releasing peptide-2 (GHRP-2) in children with GH deficiency: Growth effects and GH secretion: studies during treatment. 10th International Congress of Endocrinology, San Francisco 1996: 768.
138. Orth DN. Corticotropin-releasing hormone in humans. *Endocr Rev* 1992; **13:** 164–191.
139. Pombo M, Barreiro J, Penalva A, Mallo F, Casanueva FF, Dieguez C. Plasma growth hormone response to growth hormone releasing hexapeptide (GHRP-6) in children with short stature. *Acta Paediatr* 1995; **84:** 904–908.
140. Pombo M, Barreiro J, Penalva A, Peino R, Dieguez C, Casanueva FF. Absence of growth hormone (GH) secretion after the administration of either GH releasing hormone (GHRH), GH-releasing peptide (GHRP-6), or GHRH plus GHRP-6 in children with neonatal pituitary stalk transection. *J Clin Endocrinol Metab* 1995; **80:** 3180–3184.
141. Pong SS, Chaung LYP, Dean DC, Nargunt RP, Patchett AA, Smith RG. Identification of a new G-protein-linked receptor for growth hormone secretagogues. *Mol Endocrinol* 1996; **10:** 57–61.
142. Popovic V, Damjanovic S, Micic D, Petakov M, Dieguez C, Casanueva FF. Growth hormone (GH) secretion in active acromegaly after the combined administration of GH-releasing hormone and GH-releasing peptide-6. *J Clin Endocrinol Metab* 1994; **79:** 456–460.
143. Popovic V, Damjanovic S, Micic D, Djurovic M, Dieguez C, Casanueva FF. Blocked growth hormone-releasing peptide (GHRP-6)-induced GH secretion and absence of the synergic action of GHRP-6 plus GH-releasing hormone in patients with hypothalamopituitary disconnection: Evidence that GHRP-6 main action is exerted at the hypothalamic level. *J Clin Endocrinol Metab* 1995; **80:** 942–947.
144. Popovich V, Micic D, Damjanovich S, Djurovich M, Simic M, Gligorovic M, Dieguez C. Evaluation of pituitary GH reserve with GHRP-6. *J Pediatr Endocrinol Metab* 1996; **9:** 289–298.
145. Ramos-Dias JC, Pimentel Filho F, Reis AF, Lengyel AMJ. Different growth hormone (GH) responses to GH-releasing peptide and GH-releasing hormone in hyperthyroidism. *J Clin Endocrinol Metab* 1996; **81:** 1343–1346.
146. Renner U, Brockmeier S, Strasburger CJ, Lange M, Schopohl J, Muller OA, Werder K, Stelle GK. Growth hormone (GH)-releasing peptide stimulation of GH release from human somatotroph adenoma cells: Interaction with GH-releasing hormone, thyrotropin-releasing hormone, and octreotide. *J Clin Endocrinol Metab* 1994; **78:** 1090–1096.
147. Rolla M, Andreoni A, Bellitti D, Cristofani R, Ferdeghini M, Muller EE. Blockade of cholinergic muscarinic receptors by pirenzepine and GHRH-induced GH secretion in the acute and recovery phase of anorexia nervosa and atypical eating disorders. *Biol Psychiatr* 1991; **29:** 1079–1091.

148. Robinson BM, DeMott Friberg R, Bowers CY, Barkan AL. Acute growth hormone (GH) response to GH-releasing hexapeptide in humans in independent of endogenous GH-releasing hormone. *J Clin Endocrinol Metab* 1992; **75:** 1121–1124.

149. Robinson ICAF. Mechanism of action of GHRPs in vivo. 10th International Congress of Endocrinology, San Francisco 1996: 26.

150. Ross JRJM, Chew SL. Acquired growth hormone resistance. *Eur J Endocrinol* 1995; **132:** 655–660.

151. Rudman D, Feller AG, Nagraj HS. Effects of human growth hormone in men over 60 years old. *N Engl J Med* 1990; **323:** 1–6.

152. Sartor O, Bowers CY, Chang D. Parallel studies of His-D-Trp-Ala-Trp-D-Phe-Lys-NH2 and human pancreatic growth hormone-releasing factor-44-NH2 in rat primary pituitary cell monolayer culture. *Endocrinology* 1985; **116:** 952–957.

153. Sartor O, Bowers CY, Reynolds GA, Momany FA. Variables determining the growth hormone response of His-D-Trp-Ala-Trp-D-Phe-Lys-NH2 in the rat. *Endocrinology* 1985; **117:** 1441–1447.

154. Sartorio A, Conti A, Ferrero S, Spada A, Faglia G. GH responsiveness to repeated GHRH or Hexarelin administration in normal adults. *J Endocrinol Invest* 1995; **18:** 718–722.

155. Scheim KD, Jacks T, Cunningham P, Feeney W, Frazier EG, Niebauer GW, Zhang D, Chen H, Smith RG. Effect of MK-677 on growth hormone, IGF-I and cortisol in beagles before and after hypophysectomy. 10th International Congress of Endocrinology, San Francisco 1996: 285.

156. Sethumadhavan K, Veeraragavan K, Bowers CY. Demonstration and characterization of the specific binding of growth hormone-releasing peptide to rat anterior pituitary and hypothalamic membranes. *Biochem Biophys Res Commun* 1991; **178:** 31–37.

157. Sirinathsinghji DJS, Chen HY, Hopkins R, Trumbauer M, Heavens R, Rigby M, Smith RG, Ven der Ploeg LHT. Induction of c-fos mRNA in the arcuate nucleus of normal and mutant growth hormone-deficient mice by a synthetic non-peptydil growth hormone secretagogue. *Mol Neurosci* 1995; **6:** 1989–1992.

158. Smith RG, Cheng K, Pong SS, Hickey G, Jacks T, Butler B, Chan WWS, Chaung LYP, Judith F, Taylor J, Schoern WR, Wyvratt MJ, Fisher MH. A non-peptidyl GH secretagogue. *Science* 1993; **260:** 1640–1643.

159. Tena-Sempere M, Pinilla L, Gonzalez D, Aguilar E. Nitric oxide (NO) blocked the GH response to growth hormone releasing hormone (GHRH), growth hormone releasing hexapeptide (GHRP-6), *N*-methyl-D-aspartate (NMDA) and kainic acid (KA). 10th International Congress of Endocrinology, San Francisco 1996: 287.

160. Thomas GB, Fairhall KM, Robinson I CAF. Activation of the hypothalamo-pituitary-adrenal axis by the growth hormone (GH) secretagogue, GH-releasing peptide-6, in rats. *Endocrinology* 1997; **138:** 1585–1591.

161. Thorner MO, Vance ML, Rogol AD, Blizzard RM, Veldhuis JD, van Cauter E, Copinschi G, Bowers CY. Growth hormone-releasing hormone and growthg hormone-releasing peptide as potential therapeutic modalities. *Acta Paediatr Scand* 1990; **367**(Suppl): 29–32.

162. Thorner MO, Hartman ML, Gaylinn BD *et al.* Current status of therapy with growth hormone-releasing neuropeptides. In: Savage MO, Bourgulgon J, Grossman AB, Eds. *Front in Paediatr Neuroendocrinol*. Oxford: Blakwell Scientific, 1994: 161–167.

163. Tuilpakov AN, Bulatov AA, Peterkova A, Elizarova GP, Volevodz NN, Bowers CY. Growth hormone (GH)-releasing effects of synthetic peptide GH-releasing peptide-2 and GH-releasing hormone (1-29NH2) in children with GH insufficiency and idiopathic short stature. *Metabolism* 1995; **44:** 1199–1204.

164. Valcavi R, Dieguez C, Zini M, Muruais C, Casanueva F, Portioli I. Influence of hyperthyroidism on growth hormone secretion. *Clin Endocrinol* 1993; **38:** 515–522.

165. Vance ML, Borges JLC, Kaiser DL, Evans WS, Furlanetto R, Thominet JL, Frohman LA, Rogol AD, MacLeod M, Bloom S, Rivier J, Vale W, Thorner MO. Human pancreatic tumor

growth hormone-releasing factor (hp GRF-40): Dose–response relationship in normal man. *J Clin Endocrinol Metab* 1984; **58:** 838–845.

166. Van der Berghe G, de Zegher F, Bowers CY, Wouters P, Muller P, Soetens F, Ulasselaers D, Schetz M, Verwaest C, Lauwers P, Bouillon R. Pituitary responsiveness to GH-releasing hormone, GH-releasing peptide-2 and thyrotropin-releasing hormone in critical illness. *Clin Endocrinol* 1996; **45:** 341–351.
167. Veeragavan K, Sethumadhavan K, Bowers CY. Growth hormone-releasing peptide (GHRP) binding to porcine anterior pituitary and hypothalamic membranes. *Life Sci* 1992; **50:** 1149–1155.
168. Walker RF, Yang SW, Bercu BB. Robust growth hormone (GH) secretion in aged female rats co-administered GH-releasing hexapeptide (GHRP-6) and GH-releasing hormone (GHRH). *Life Sci* 1991; **49:** 1499–1504.
169. Walker RF, Yang AW, Masuda R, Hu CS, Bercu BB. Effects of growth hormone releasing peptides on stimulated growth hormone secretion in old rats. In: Bercu BB, Walker RF, Eds. *Growth hormone II, Basic and Clinical Aspects.* Springer-Verlag, New York, 1993: 167–192.
170. Wu D, Chen C, Katoh K, Zhang J, Clarke J. The effect of GH-releasing peptide-2 (GHRP-2 or KP 102) on GH secretion from cultured ovine pituitary cells can be abolished by a specific GH-releasing factor (GRF) receptor antagonist. *J Endocrinol* 1994; **140:** 9–13.
171. Wu D, Chen C, Zhang J, Katoh K, Clark I. Effects in vitro of new growth hormone releasing peptide (GHRP-1) on growth hormone secretion from ovine pituitary cells in primary culture. *J Neuroendocrinol* 1994; **6:** 185–190.
172. Yagi H, Kaji H, Sato M, Okiimura Y, Chihara K. Effect of intravenous or intracerebroventricular injections of His-D-Trp-Ala-Trp-D-Phe-Lys-NH2 on GH release in conscious, freely moving male rats. *Neuroendocrinology* 1996; **63:** 198–206.