Metabolic Modulation of the Growth Hormone–Releasing Activity of Hexarelin in Man

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Hexarelin (His-D-2-methyl-Trp-Ala-Trp-D-Phe-Lys-NH_2) is a new potent synthetic growth hormone (GH)-releasing hexapeptide. The mechanism of action of hexarelin in man has never been evaluated. Hexarelin may act directly on specific pituitary receptors and indirectly on the hypothalamus. To elucidate its mechanism of action in man, we studied the interaction of hexarelin with glucose and free fatty acids (FFA), two metabolic factors known to inhibit both basal and GH-releasing hormone (GHRH) stimulated GH secretion. Glucose is thought to inhibit GH secretion via stimulation of endogenous somatostatin release, whereas FFA could also act directly on somatotrope cells. Therefore, we investigated the effect of oral glucose (100 g) and lipid-heparin infusion (250 mL of a 10% lipid solution + 2,500 U heparin) on the GH response to a maximal dose (2 μg/kg intravenously [IV]) of hexarelin or GHRH in six normal men. Hexarelin elicited a clear-cut GH response (mean ± SEM; peak, 62.6 ± 8.0 μg/L) that was higher ($P < .01$) than that observed after GHRH (peak, 19.8 ± 2.4 μg/L). Although similar increases in plasma glucose were observed with the two peptides, oral glucose almost abolished the GH response to GHRH (peak, 5.6 ± 0.9 μg/L, $P < .01$) while only blunting the somatotrope response to hexarelin (peak, 38.4 ± 7.9 μg/L, $P < .05$). Similarly, lipid-heparin infusion nearly abolished the GH response to GHRH (peak, 4.9 ± 1.0 μg/L, $P < .01$) while only blunting the somatotrope response to hexarelin (peak, 34.2 ± 4.5 μg/L, $P < .05$). This study shows that hexarelin releases more GH than GHRH and that it is more resistant than GHRH to the inhibitory effect of glucose or FFA. Its resistance to inhibitory influences could be due to antagonism of somatostatinergic activity within the hypothalamus or directly at the pituitary level, although unknown mechanisms cannot be ruled out.

GROWTH HORMONE (GH) secretion is mainly regulated by two neurohormones, GH-releasing hormone (GHRH) and somatostatin.[1] However, insulin-like growth factor-I, neurotransmitters, neuropeptides, hormones, and metabolic variables also have an important influence on somatotrope secretion.[1]

It is well known that metabolic variables such as glucose and free fatty acids (FFA) inhibit both basal and GHRH-stimulated GH release in man.[2-5] The inhibitory effect of glucose on GH secretion is believed to be mediated by the stimulation of somatostatin release from the hypothalamus.[3,6] The inhibitory activity of FFA on GH release takes place directly at the pituitary level,[7,8] although a stimulatory effect on hypothalamic somatostatin has also been hypothesized.[9,10]

GH-releasing peptides (GHRPs) are a new class of short synthetic peptides[11] derived from enkephalin. They are devoid of opioid activity[12,13] and have no primary sequence homology with GHRH.[13,14] In vivo, GHRPs release more GH than GHRH.[15,16] They have specific, non-GHRH, non-opioid receptors in both the hypophysis and the hypothalamus,[17-22] suggesting that they mimick endogenous substances. These small peptides probably act at the pituitary level, although a simultaneous action on the hypothalamus has been proposed.[15-23]

Hexarelin is a new synthetic hexapeptide.[24,25] Its chemical structure (His-D-2-methyl-Trp-Ala-Trp-D-Phe-Lys-NH_2) is similar to that of GHRP-6, the most widely studied GHRP. Compared with GHRP-6, hexarelin is more stable under a number of degradation conditions.[26] Studies in rats have shown that the drug is more potent than GHRP-6.[26,27] In man, the GH response to hexarelin administered intravenously (IV) is much higher than that to GHRH and has a limited variability.[28-30] The drug is also active by subcutaneous, intranasal, and oral routes.[29]

To elucidate its mechanism of action in man, we studied the interaction of hexarelin with glucose and FFA. Specifically, we investigated whether the GH-releasing effect of this peptide is reduced by metabolic inputs known to inhibit strongly both basal and GHRH-stimulated GH secretion in man.[3-7]

SUBJECTS AND METHODS

Treatments

Vials containing 100 μg lyophilized hexarelin were kindly provided by Europeptides (Argenteuil, France). Vials containing 50 μg lyophilized $GHRH_{1-29}$ (Geref) were purchased from Serono (Milan, Italy). Hexarelin or GHRH were dissolved in 2 mL isotonic saline to be administered as IV boluses. Bottles containing 250 mL lipid-heparin solution (Intralipid) were purchased from Kabi PH-Pierrel (Milan, Italy). Intralipid is a 10% lipid solution with 2,500 U heparin. Bottles containing 100 g glucose in 150 mL of a 33% solution were provided by our hospital's pharmacy.

Study Design

Six normal men (aged 26 to 27 years) volunteered for the study. Their mean body weight was 70.0 ± 11.9 kg, and all were within 20% of their ideal body weight. All subjects gave their written informed consent before taking part in the study. Subjects underwent six treatment sessions separated by a wash-out period of at

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least 3 days. Subjects were admitted to the clinical unit on the morning of treatment administration following an overnight fast. Hexarelin and GHRH were administered between 8 and 9 AM. The cubital veins were cannulated 30 minutes before drug administration and kept patent by slow infusion of isotonic saline.

All subjects received the following treatments during six different sessions: hexarelin (2 μg/kg IV at 0 minutes); GHRH (2 μg/kg IV at 0 minutes); hexarelin (2 μg/kg IV at 0 minutes) + glucose (100 g orally at −45 minutes); hexarelin (2 μg/kg IV at 0 minutes) + lipid-heparin infusion (250 mL of a 10% lipid solution + 2,500 U heparin from −30 to +120 minutes); GHRH (2 μg/kg IV at 0 minutes) + glucose (100 g orally at −45 minutes); and GHRH (2 μg/kg IV at 0 minutes) + lipid-heparin infusion (250 mL of a 10% lipid solution + 2,500 U heparin from −30 to +120 minutes). The doses of hexarelin (2 μg/kg) and GHRH (2 μg/kg) were chosen based on literature showing that both are maximal doses.[30,31]

Blood samples were taken every 15 minutes from −60 to +120 minutes. Serum GH levels were measured in duplicate with an immunoradiometric assay (hGH-CTK, Sorin Biomedica, Saluggia, Italy). All samples from the same subject were analyzed together. Assay sensitivity was 0.15 μg/L, and the ranges of interassay and intraassay coefficients of variation were 4.9% to 6.5% (2 to 40 μg/L) and 1.5% to 2.9% (2 to 40 μg/L), respectively. GH secretory responses are expressed either as absolute values (micrograms per liter) or as areas under the curve of GH plasma levels from 0 to 120 minutes ([$AUC_{0\text{-}120}$] micrograms per minute per liter) calculated by trapezoidal integration.

Plasma glucose levels (milligrams per 100 milliliters) were measured during treatment with hexarelin or GHRH alone and combined with oral glucose. The same time points as used for GH determinations were used. Plasma was analyzed with a glucose oxidase method (Beckman Glucose Analyzer 2, Beckman Instruments, Brea, CA).

Plasma FFA levels (milliequivalents per liter) were measured during treatment with hexarelin or GHRH alone and combined with lipid-heparin infusion. Measurements were performed every 30 minutes from −60 to +120 minutes. An enzymatic assay using the NEFA QUICK BMY kit (Boehringer Mannheim/Yamanouchi K.K., Tokyo, Japan) was used. Glucose or FFA plasma levels are expressed as absolute values.

Statistical Analysis

Statistical comparisons were performed with nonparametric tests. The Friedman test was used to compare simultaneously the different treatments, and the Dunn test was used for multiple pairwise comparisons. P values less than .05 were considered statistically significant (two-sided test). Calculations were performed with the SigmaStat statistical package.[32] Although nonparametric tests were used for statistical comparisons, results are expressed as the mean ± SEM.

RESULTS

There were no significant differences in basal GH levels during the six treatment sessions. Hexarelin induced a much higher GH response (peak, 62.6 ± 8.0 μg/L; $AUC_{0\text{-}120}$, 4,586 ± 674 μg · min/L) than GHRH (peak, 19.8 ± 2.4 μg/L; $AUC_{0\text{-}120}$, 1,305 ± 65 μg · min/L, $P < .01$; Fig 1).

The increase in plasma glucose after the oral glucose load observed during hexarelin treatment (from 79.3 ± 1.3 mg/100 mL at −60 minutes to 134.5 ± 5.9 at −15 minutes, $P < .01$) was similar to that during GHRH treatment (from 78.7 ± 1.8 mg/100 mL at −60 minutes to 136.5 ± 5.4 at −15 minutes, $P < .01$). The GH-releasing effect of GHRH was markedly inhibited by glucose (peak, 5.6 ± 0.9 μg/L; $AUC_{0\text{-}120}$, 391 ± 61 μg · min/L; $P < .01$), whereas that of hexarelin was only blunted (peak, 38.4 ± 7.9 μg/L; $AUC_{0\text{-}120}$, 2,237 ± 564 μg · min/L, $P < .05$; Fig 2).

Lipid-heparin infusion reproducibly increased plasma FFA during both the hexarelin (from 0.38 ± 0.06 mEq/L at −60 minutes to 2.80 ± 0.29 at +60 minutes, $P < .05$) and GHRH treatments (from 0.42 ± 0.06 mEq/L at −60 minutes to 2.66 ± 0.16 at +60 minutes, $P < .05$). Basal GH levels were reduced in both treatments (from 0.9 ± 0.8 μg/L at −60 minutes to 0.5 ± 0.4 at 0 minutes and from 1.0 ± 0.5 at −60 minutes to 0.2 ± 0.1 at 0 minutes for hexarelin and GHRH, respectively). The GH-releasing effect of GHRH was nearly abolished (peak, 4.9 ± 1.0 μg/L; $AUC_{0\text{-}120}$, 237 ± 31 μg · min/L; $P < .01$), whereas that of hexarelin was only attenuated (peak, 34.2 ± 4.5 μg/L; $AUC_{0\text{-}120}$, 2,141 ± 253, $P < .05$; Fig 3).

The GH response to hexarelin after glucose (Fig 2) was similar to that during lipid-heparin infusion (Fig 3), and both were still much higher than the GH response obtained after GHRH alone ($P < .05$). Oral glucose or lipid-heparin infusion did not cause variations in the time of occurrence of the peak GH response to hexarelin or GHRH.

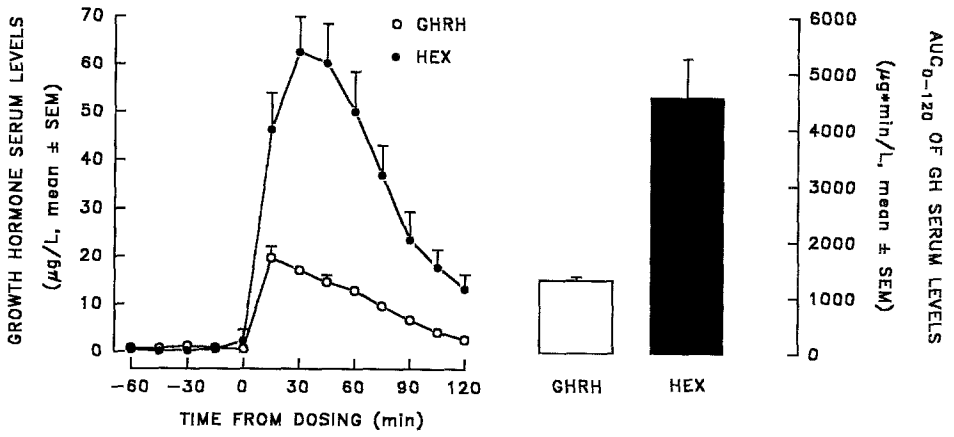

Fig 1. GH responses to hexarelin ([HEX] 2 μg/kg IV) or GHRH (2 μg/kg IV) in six healthy men.

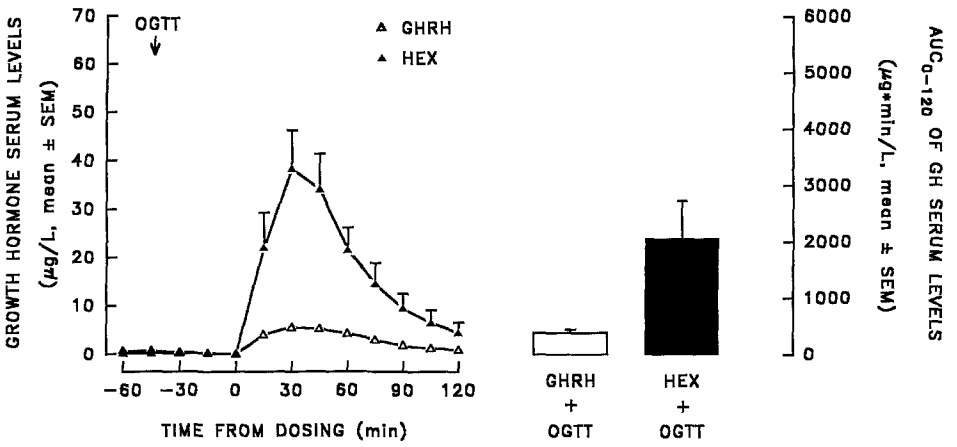

Fig 2. GH responses to HEX (2 μg/kg IV) or GHRH (2 μg/kg IV) administered in combination with oral glucose (100 g) in six healthy men. OGTT, oral glucose tolerance test.

Adverse Events

A transient facial flushing was observed in two subjects after administration of hexarelin and in five subjects after GHRH. The oral glucose load and lipid-heparin infusion elicited no adverse events.

DISCUSSION

This study shows that although oral glucose and lipid-heparin infusion only blunt the potent GH-releasing effect of hexarelin, they nearly abolish the somatotrope response to GHRH. Previous studies on GHRH have reported similar results.[3,5-7] To our knowledge, no data have been obtained in man on the interaction between glucose or FFA and other GHRPs.

The mechanism of action of GHRPs is not fully understood. It is believed that these small peptides act directly at the pituitary level on specific non-GHRH, non-opiate receptors.[11-22] In vitro, GHRPs stimulate both basal and GHRH-induced GH secretion from rat pituitary.[17-21] This stimulating effect is much greater in vivo,[11,12,15,16,23,33,34] suggesting that these peptides also act on the hypothalamus, where specific receptor sites have been found.[22]

Although the activity of GHRH-secreting neurons seems to be necessary to produce the GH-releasing effect of GHRPs,[15,23,33,34] it is unlikely that they stimulate GHRH release. In fact, their combined administration with GHRH induces a synergistic effect on GH secretion.[11,34,35] Other data indicate that GHRPs do not inhibit hypothalamic somatostatin release. Both somatostatin antiserum[15,23] and antisomatostatin agents potentiate the GH-releasing effect of GHRPs.[16,36] Studies in man indicating that somatostatin only blunts the GH response to hexarelin[35] confirm animal data showing that GHRPs may antagonize the inhibitory effect of somatostatin on somatotrope cells.[21]

The inhibitory effect of acute hyperglycemia on GH secretion in man is well known. Glucose inhibits both physiologically elevated basal GH levels and the somatotrope response to different stimuli, including GHRH.[2,3] Conversely, basal and GHRH-stimulated GH secretion from rat anterior pituitary cells are unaffected in vitro by different glucose concentrations.[6] Thus, it is unlikely that glucose acts directly on the pituitary. As demonstrated by the present study and others,[3,37-39] glucose markedly inhibits the GH response to the maximal GHRH dose. Thus, it is also unlikely that glucose modulates the activity of GHRH-secreting neurons. Studies in man suggest that substances such as arginine and pyridostigmine known to inhibit somatostatin release counteract the inhibitory effect of acute hyperglycemia on both basal and GHRH-stimulated GH secretion.[37-39] This would support the hypothesis that

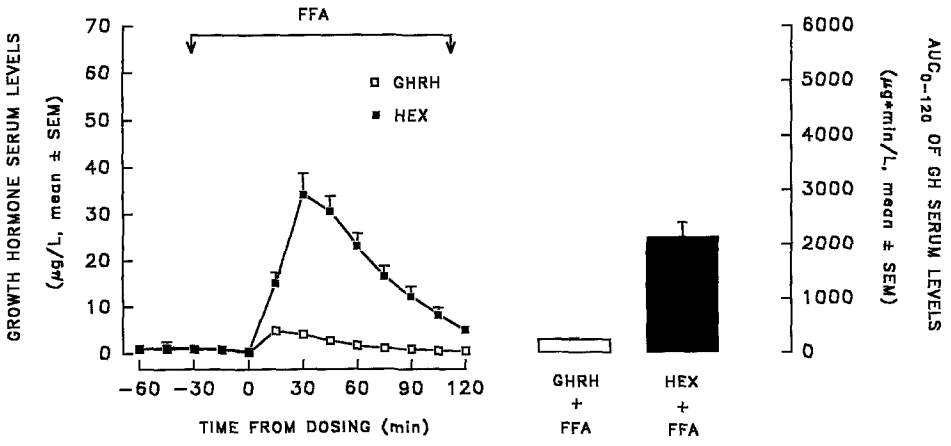

Fig 3. GH responses to HEX (2 μg/kg IV) or GHRH (2 μg/kg IV) administered in combination with lipid-heparin infusion (250 mL of a 10% lipid solution + 2,500 U heparin) in six healthy men.

glucose has a stimulatory effect on hypothalamic somatostatin. Also, studies in the rat suggest that the effect of glucose may be mediated by somatostatin,[40,41] stimulating rather than inhibiting GH secretion. The present results showing that the GH response to hexarelin is blunted but not abolished by glucose indicate that in man the stimulating effect of GHRPs is partially resistant to an increase in endogenous somatostatin. Indeed, GHRPs may antagonize the hyperpolarizing effect of somatostatin on the somatotrope cell membrane.[21] However, these peptides might also counteract the effect of glucose on somatostatinergic neurons at the hypothalamic level. Indeed, hexarelin enhances GHRH-stimulated GH release.[35]

The potent inhibitory effect of FFA on both basal and GHRH-induced GH secretion may be explained by a direct action on the pituitary. The inhibitory effect of FFA on GH responses to GHRH has been observed both in vitro[42,43] and in vivo in hypophysectomized-transplanted rats.[44] In man, FFA counteract the GH response to both arginine and pyridostigmine.[45,46] On the other hand, in the rat somatostatin antiserum counteracts the inhibitory effect of FFA on GH release.[41] In the monkey,[10] the intraventricular injection of FFA inhibits GH secretion. These studies suggest that the inhibitory effect of FFA is mediated by stimulation of hypothalamic somatostatin release. However, recent studies in the rat indicate that FFA inhibit somatostatin release from the hypothalamus.[8] The present study shows that the GH-stimulating effect of hexarelin in man is partially resistant to the inhibitory effect of FFA. These results differ from those obtained in the rat with GHRP-6,[47] but support the finding that GHRP-6 counteracts the inhibition of calcium uptake by FFA at the pituitary level.[48]

In conclusion, the present study demonstrates that, unlike GHRH, the GH-releasing effect of hexarelin is partially resistant to the strong inhibitory effect of glucose or FFA. This and the strong activity of hexarelin make it a candidate for restoring the function of the GH–insulin-like growth factor-I axis in patients with GH insufficiency due to hypothalamic pathogenesis.

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