

Hexarelin-Induced Growth Hormone, Cortisol, and Prolactin Release: A Dose-Response Study

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ABSTRACT

Dose-response data for GH-releasing peptides are limited. We studied the effects of varying doses (0–1.0 $\mu\text{g}/\text{kg}$) of hexarelin, a novel GH-releasing peptide, administered iv to healthy adult males on GH, PRL, and cortisol release. In addition, we studied the effect of administration of a single dose of GHRH-(1–29)-NH₂ (1.0 $\mu\text{g}/\text{kg}$), alone or in combination with a low dose of hexarelin (0.125 $\mu\text{g}/\text{kg}$). Dose-response curves for the maximum GH response and maximum percent change in serum PRL and cortisol concentrations from baseline were constructed.

The GH dose-response curve reached a plateau of 140 mU/L, corresponding to a hexarelin dose of 1.0 $\mu\text{g}/\text{kg}$, with an ED₅₀ of 0.48 \pm 0.02 $\mu\text{g}/\text{kg}$ (mean \pm SEM). The PRL dose-response curve reached a

plateau of 180% for the maximum percent rise from baseline, corresponding to a hexarelin dose of 1.0 $\mu\text{g}/\text{kg}$, with an ED₅₀ of 0.39 \pm 0.02 $\mu\text{g}/\text{kg}$. The cortisol dose-response curve showed a step increase to approximately 40% at a hexarelin dose of 0.5 $\mu\text{g}/\text{kg}$. The coadministration of GHRH-(1–29)-NH₂ (1.0 $\mu\text{g}/\text{kg}$) and low dose hexarelin (0.125 $\mu\text{g}/\text{kg}$) resulted in massive GH release (115 \pm 32.8 mU/L), a moderate rise in serum PRL (84.9 \pm 27.5%), and no rise in serum cortisol.

These data show that iv hexarelin was capable of inducing GH, PRL, and cortisol release in a dose-dependent manner. Low dose hexarelin was synergistic with GHRH and potent for GH release with a minimal effect on other hormones. (*J Clin Endocrinol Metab* 81: 4338–4341, 1996)

HEXARELIN (His-D-2-methyl-Trp-Ala-Trp-D-Phe-Lys-NH₂) is a novel GH-releasing peptide (GHRP) capable of massive GH secretion after iv, sc, intranasal, and oral administration and is well tolerated in man (1, 2). It is an analog of GHRP-6 in which the D-tryptophan has been substituted for its 2-methyl derivative. The GH-releasing activity of hexarelin and other GHRPs has been extensively studied in both health and disease states (2–8). The high potency and low toxicity of these peptides together with their oral activity have made them likely candidates for future therapeutic uses in GH deficiency states.

Most studies of GHRPs have been conducted using 1.0 μg peptide/kg BW, given iv, a dose capable of generating high serum GH concentrations (2–5, 9–13). GH concentrations of such magnitude are not needed for normal human growth, as evident from the physiological pattern of GH secretion (14–16). Lower doses of GHRPs are likely to be used in future therapeutic interventions, provided they are capable of releasing adequate GH from the pituitary. Dose-response data on GHRPs are limited (17–19) and do not adequately address this issue.

In a previous study (20) we observed that hexarelin was not specific for GH release when administered iv to healthy adults. Significant elevation of serum cortisol and PRL occurred after the administration of 1.0 $\mu\text{g}/\text{kg}$ boluses, an observation made by others and not unique to hexarelin (3, 18). The nonspecificity of hexarelin and other GHRPs for GH

release is of concern if these agents are to be used in future clinical trials.

To address these issues we have constructed dose-response relationships for GH, cortisol, and PRL. In addition, to minimize the effect of hexarelin on cortisol and PRL without compromising its GH-releasing potential, we have investigated the effect of low dose hexarelin alone or in combination with GHRH.

Subjects and Methods

Subjects

Six healthy adult males, aged 20.6–35.1 yr, with normal body mass index (median, 22.5 kg/m²; range, 19.8–28.9) were studied. All six were screened for symptoms and signs of endocrine disorders. None was receiving medication during the study period. Written informed consent was obtained. The study was approved by the ethics committee of Middlesex Hospital.

Study design

After an overnight fast, subjects were admitted to the clinical unit and remained in a recumbent position throughout the study session. Two indwelling iv cannulas were inserted in the forearms at 0830 h (–60 min). One cannula was used for drug administration, and the other for collection of blood specimens. Blood samples for measurement of serum GH, PRL, cortisol, TSH, insulin, and blood glucose were drawn at 15-min intervals from 0900 h (–30) to 1130 h (120 min). At 0930 h (0 min), a bolus of saline, GHRH-(1–29)-NH₂ (1.0 $\mu\text{g}/\text{kg}$; Pharmacia, Stockholm, Sweden), or varying dosages of hexarelin (Europeptides, Argenteuil, France) were administered iv (studies 1–8; Fig. 1).

The studies were conducted in a random order, with a washout period of at least 72 h between each, although in the majority of cases the interval was 7 days or more, and were completed by all six subjects. After analysis of the data from studies 1–8, one additional study was conducted using a combination of GHRH-(1–29)-NH₂ (1.0 $\mu\text{g}/\text{kg}$) and low dose hexarelin (0.125 $\mu\text{g}/\text{kg}$; study 9; Fig. 1). These were completed by four of the six subjects originally recruited.

Samples for hormone measurement from each study were spun at 4

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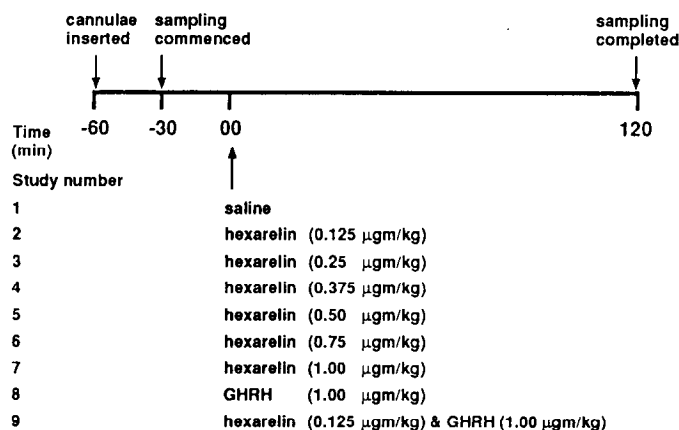


FIG. 1. Study design. An iv bolus of 0.9% saline (study 1) or varying doses of hexarelin (studies 2–7), GHRH-(1–29)-NH₂ (study 8), or hexarelin with GHRH-(1–29)-NH₂ was administered at time zero. The cannulas were inserted at –60 min; sampling was commenced at –30 min and completed at 120 min. Specimens were collected at 15-min intervals.

C, separated, and stored at –20 C until assayed. Samples for blood glucose measurement were analyzed on the day of collection.

Assays

Serum GH concentrations were measured using an immunoradiometric assay (Hybritech Tandem-R hGH kit, Hybritech, Liege, Belgium). The sensitivity of the assay was 0.5 mU/L. Intraassay coefficients of variation were 10.6%, 4.9%, 5.2%, 4.9%, and 5.0% at serum GH concentrations of 1.4, 3.5, 14.4, 26.4, and 99.4 mU/L, respectively; interassay coefficients of variation were 10.5%, 7.2%, and 5.4% at concentrations of 6.0, 13.2, and 33.3 mU/L, respectively. The standard used was HS2443E (NIH), which had been recalibrated to milliunits per L (1 ng/mL = 2.6 mU/L) with the International Standard 80/505. The assay did not cross-react with PRL.

Serum PRL concentrations were measured using an immunoradiometric assay (NETRIA, St. Bartholomew's Hospital, London, UK). The sensitivity of the assay was 10 mU/L. Intraassay coefficients of variation were 2.5%, 1.4%, and 2.6% at serum PRL concentrations of 165, 562, and 1159 mU/L, respectively; interassay coefficients of variation were 8.3%, 5.1%, and 7.9% at concentrations of 173, 506, and 1103 mU/L, respectively. The assay did not cross-react with GH.

Serum cortisol concentrations were measured using a solid phase RIA (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA). The sensitivity of the assay was 5.5 nmol/L. Intraassay coefficients of variation were 5.7%, 3.1%, and 2.6% at serum cortisol concentrations of 27.6, 96, and 552 nmol/L, respectively; interassay coefficients of variation were 6.3% and 4.5% at concentrations of 138 and 276 nmol/L, respectively.

Serum TSH concentrations were measured using a solid phase immunoradiometric assay kit (NETRIA, St. Bartholomew's Hospital), serum insulin concentrations were measured using a commercial RIA (Diagnostic Systems Laboratories, Webster, TX), and whole blood glucose concentrations were measured using a Yellow Springs Industrial Analyzer model 23 (Yellow Springs, OH).

Statistics

The peak serum GH concentrations for each subject after the administration of iv saline and varying dosages of hexarelin were used to construct the GH dose-response curve, using the curve-fitting function of the statistics package SPSS. The mean and SEM of the ED₅₀ (the dose that would be expected to produce 50% of the maximal response) were obtained by curve fitting each subject's peak serum GH concentration after the administration of iv saline or varying doses of hexarelin, plotted against the dose administered.

The data for serum PRL and cortisol concentrations were analyzed on the basis of the maximum percent change after the administration of saline or varying doses of hexarelin compared with baseline values

measured at 0 min. The data thus obtained were then used to construct the dose-response curves for each hormone. The mean and SEM of the ED₅₀ for the maximum change in serum PRL concentration were obtained using the method outlined above.

The rationale for using the change in hormone concentration as opposed to the absolute measured concentration is that, unlike GH, PRL and cortisol levels are detectable in the blood throughout the day and have a wide range of normal values with considerable interindividual variation.

Curve fitting was not possible for the cortisol data, which are presented as a dot plot.

Results

GH dose-response curve

The curve constructed is shown in Fig. 2. The curve reached a plateau at a serum GH concentration of approximately 140 mU/L, corresponding to a hexarelin dose of 1.0 µg/kg. The best-fit GH dose-response curve for all subjects was described by the cubic equation: $y = 0.39 + 47.4(\text{hexarelin dose}) + 264.9(\text{hexarelin dose})^2 - 182(\text{hexarelin dose})^3$, where y is the serum GH concentration. The ED₅₀ was 0.48 ± 0.02 µg/kg (mean \pm SEM). A hexarelin dose as low as 0.2 µg/kg would be expected to generate a peak GH response of 20 mU/L.

PRL dose-response curve

The PRL dose-response curve is shown in Fig. 3. The curve reached a plateau at approximately 180%. The maximum percent rise in the PRL concentration from baseline values at 0 min corresponds to a hexarelin dose of 1.0 µg/kg. The ED₅₀ was 0.39 ± 0.02 µg/kg (mean \pm SEM). The best-fit curve for the maximum change in PRL concentration for all subjects was described by the cubic equation: $y = 25.6 - 160.6(\text{hexarelin dose}) + 1291.9(\text{hexarelin dose})^2 - 1005.5(\text{hexarelin dose})^3$, where y is the serum PRL concentration.

Cortisol dose-response curve

The dot plot of increasing doses of iv hexarelin vs. the maximum percent change in serum cortisol concentration compared to baseline values at 0 min (Fig. 4) showed no clear

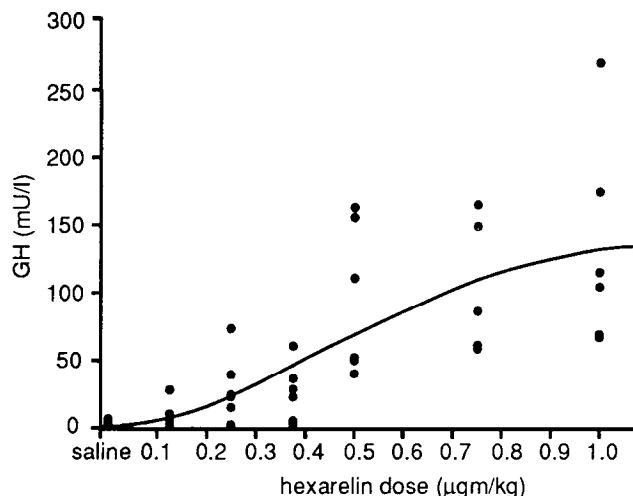


FIG. 2. Dose-response curve of iv hexarelin dose plotted on the x-axis vs. serum GH concentration plotted on the y-axis. The S-shaped curve reached a plateau at a GH concentration of 140 mU/L.

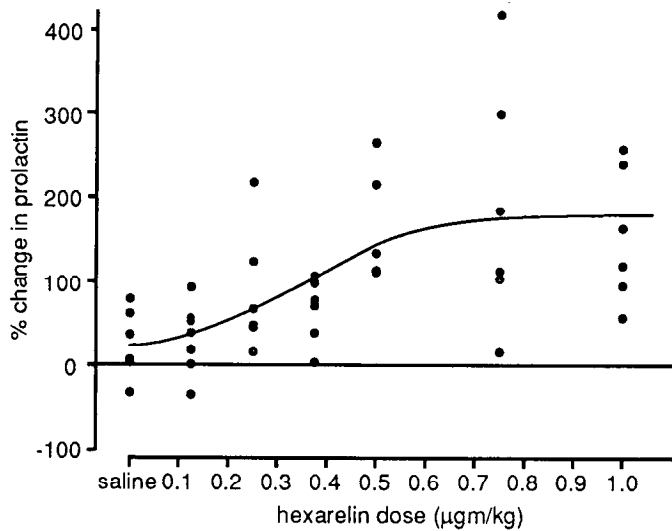


FIG. 3. Dose-response curve of iv hexarelin plotted on the x-axis vs. the maximum percent change in the serum PRL concentration from the baseline value at 0 min plotted on the y-axis. The S-shaped curve reached a plateau at a maximum change of 180%.

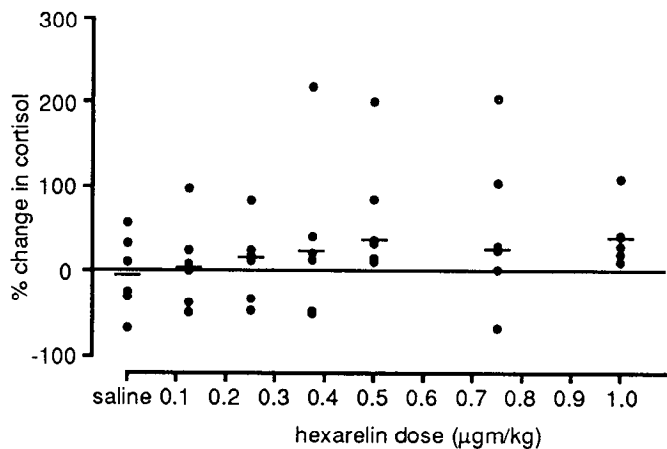


FIG. 4. Dose-response of iv hexarelin plotted on the x-axis vs. maximum percent change in the serum cortisol concentration from the baseline value at 0 min plotted on the y-axis. The dot plot shows a step to a new plateau of a 40% rise in cortisol concentration at a dose of 0.5 µg/kg. Median values are represented by horizontal bars.

dose-response effect. The maximum percent changes in the serum cortisol concentration after the iv administration of low doses of hexarelin (≤ 0.375 µg/kg) were equally distributed around the zero line, with no clear-cut rise or fall in concentration, although the median values were slightly positive. However, at higher doses (≥ 0.5 µg/kg) the change was predominantly an increase. The data could be described as a step change to a new plateau of maximum percent change in serum cortisol concentration. An approximate 40% step change occurred at a hexarelin dose of 0.5 µg/kg.

Serum TSH, serum insulin, and blood sugar

Serum TSH, serum insulin, and blood sugar concentrations did not change in response to hexarelin administration. The maximum changes in serum TSH, serum insulin, and blood sugar concentrations were (mean \pm SEM) 0.1 ± 0.2 mU/L at a hexarelin dose of 0.375 µg/kg, 1.7 ± 2.7 IU/L at

a hexarelin dose of 0.25 µg/kg, and -0.4 ± 0.3 mmol/L at a hexarelin dose of 0.125 µg/kg, respectively.

GHRH and combined GHRH and hexarelin studies

The peak serum GH responses to GHRH-(1-29)-NH₂ (1.0 µg/kg), hexarelin (0.125 µg/kg), and GHRH-(1-29)-NH₂ (1.0 µg/kg) with hexarelin (0.125 µg/kg) were 42.5 ± 7.8 , 7.9 ± 4.1 , and 115 ± 32.8 mU/L, respectively. The corresponding maximum percent changes in serum PRL concentrations were $72.0 \pm 23.3\%$, $28.5 \pm 17.8\%$, and $84.9 \pm 27.5\%$, respectively. There was no rise in serum cortisol concentration after the administration of GHRH-(1-29)-NH₂ (1.0 µg/kg) alone or in combination with low dose hexarelin (0.125 µg/kg), and there was only a small rise after low dose hexarelin (0.125 µg/kg) alone (mean \pm SEM, $7.6 \pm 21.2\%$).

Discussion

This study shows that iv hexarelin is capable of inducing GH release in a dose-dependent manner in healthy adult males and that hexarelin is a nonspecific GH secretagogue, inducing concomitant increases in serum PRL and cortisol levels. The rise in serum PRL was dose related, whereas that in cortisol occurred beyond a threshold dose of hexarelin. Intravenous hexarelin had no effect on blood sugar, serum insulin, or serum TSH concentrations. The combined iv administration of GHRH-(1-29)-NH₂ with low dose hexarelin restored its massive GH-releasing ability and resulted in only a modest elevation of PRL levels, with no effect on cortisol secretion.

Most studies of hexarelin have been conducted using high doses (1.0 µg/kg), which are known to induce massive GH release. Such levels are not needed for normal linear growth. Doses of hexarelin capable of generating lower, but adequate, levels of GH in the blood are more likely to be used in therapy because they would mimic the physiological levels of serum GH essential for normal growth. Our data show that doses of hexarelin as low as 0.2 µg/kg are capable of generating serum GH concentrations equivalent to the maximum seen in normal adults during the day (21, 22) and would be expected to generate at least similar levels in children.

The GH dose-response curve showed a plateau at a dose of 1.0 µg/kg, suggesting that such a dose was near-maximal. A dose response study by Imbimbo *et al.* (19) in which up to 2.0 µg/kg were administered to normal adults showed that increasing the dose of hexarelin from 1 to 2 µg/kg resulted in only an additional 6% increase in the serum GH concentration, suggesting that the maximal GH response to hexarelin occurred between 1–2 µg/kg. Establishing the maximal and near-maximal doses of hexarelin is important because it may have a role as a diagnostic test (23), and the level is useful for comparison with other tests of pituitary GH reserve, such as the GHRH test, in which the use of maximal to supramaximal doses is standard practice for testing the readily available pool of GH (24).

The concomitant increases in serum PRL and cortisol after treatment with iv hexarelin indicates, contrary to earlier beliefs (17, 25), that GHRPs are not as specific GH secretagogues as was first thought. The absence of a hexarelin effect

on serum TSH levels argues against the effect on PRL being TRH mediated. Besser *et al.* (26) documented this nonspecificity of hexarelin and found that the cortisol release was accompanied by a rise in ACTH. These data suggest that hexarelin acts at receptor sites not purely confined to the somatotroph. Although the maximum levels to which serum PRL and cortisol concentrations rose remained within the normal range, and the values returned to baseline levels by 120 min, these findings may have far reaching implications in terms of the potential use of GHRPs in the therapeutic setting. The prolonged and repeated use of GHRPs may result in hypercortisolemic and/or hyperprolactinemic states if such effects on PRL and cortisol release are sustained. Our study has only addressed these effects in the acute setting, so the effects of chronic administration of hexarelin on serum PRL and cortisol remain unknown. It is prudent to mention that to date there have been no reports of orally administered GHRPs resulting in significant PRL release (27, 28). The reasons for this are unclear, but may be related to its bioavailability after oral ingestion.

The finding that the hexarelin-induced rise in serum PRL and cortisol is dose dependent, coupled with its dose-dependent GH release, has enabled us to identify a dose of hexarelin (0.2 $\mu\text{g}/\text{kg}$) that results in adequate GH release with minimal effect on serum cortisol and PRL.

The synergistic GH-releasing effect of GHRH and GHRPs, using near-maximal doses, is well documented (27). The use of low dose GHRP with a maximal dose of GHRH is also known to be synergistic (27). Our study shows that the combination of GHRH and low dose hexarelin induces massive GH release with no effect on serum cortisol and only a modest elevation of serum PRL levels. Furthermore, using a combination of GHRH and low dose hexarelin restored and exceeded the massive GH release observed with hexarelin alone. This opens up the possibility of using even smaller doses of GHRP in combination with GHRH. The obvious route of GHRP administration would be oral, given perhaps twice daily, with GHRH given as a depot preparation.

This study provides important dose-response data for hexarelin's GH-, PRL-, and cortisol-releasing properties. It provides further confirmation of the synergistic action of low dose GHRP and GHRH and the potential advantage of applying this approach in future trials.

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