Effects of repeated doses and continuous infusions of the growth hormone-releasing peptide hexarelin in conscious male rats

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Abstract

We have previously shown that hexarelin, a novel GH-releasing peptide (GHRP), is able to elicit GH release when administered i.v., s.c. or by mouth and that it is a more potent GH secretagogue than GHRP-6. In the current study, we investigated the effects of hexarelin administered as repeated doses at 2 h intervals or as a continuous 6, 30 or 174 h infusion to conscious male rats.

In the first experiment, adult male Sprague–Dawley rats were prepared with dual indwelling jugular catheters. On the day of experimentation, these animals received three 25 μg/kg i.v. boluses of hexarelin at 2 h intervals with blood sampling at 5, 10, 15, 30, 60, 90 and 120 min after each dose. The mean peak GH response and the mean area under the GH response curve (AUC) for the 30 min after each administration were calculated and are reported as the mean ± S.E.M. For both the peak and AUC results there was a significant ($P<0{\cdot}05$) difference in the GH response noted between the first (peak 301 ± 37 ng/ml; AUC 5585 ± 700 ng/ml per 30 min) and second (peak 149 ± 47 ng/ml; AUC 3056 ± 908 ng/ml per 30 min) injections of hexarelin, but not between the first and third (peak 214 ± 49 ng/ml; AUC 3862 ± 844 ng/ml per 30 min). In a second series of experiments, adult male Sprague–Dawley rats received continuous infusions (100 μg/h) of hexarelin or saline (1 ml/h) for 6, 30 or 174 h. Blood samples were collected every 20 min for the duration of the 6 h infusion and for the last 6 h of the two longer hexarelin infusions. Plasma GH concentrations peaked within 40 min of the initiation of infusion, but soon returned to basal levels. Mean plasma GH concentrations did not differ between any of the treatment groups, nor did any of the parameters of pulsatile hormone release analyzed. No significant differences in plasma corticosterone concentrations were noted between any of the treatment groups. On the other hand, while neither the 6 h (941 ± 70 ng/ml) nor the 30 h (954 ± 70 ng/ml) hexarelin infusions resulted in a significant increase in the plasma IGF-I concentrations over those noted in the saline controls (935 ± 65 ng/ml), a 174 h hexarelin infusion did elicit a significant increase (1289 ± 42 ng/ml; $P<0{\cdot}05$). Thus it appears that, while continuous exposure to hexarelin does not disrupt normal GH cycling, it may (after up to 174 h of exposure) alter other components of the growth axis. In addition, since the character of pulsatile GH release remained unaltered in response to the hexarelin infusion, it appears that this GHRP may not act by suppression of functional somatostatin tone as has been suggested previously.

Journal of Endocrinology (1998) **158,** 367–375

Introduction

Since the discovery of the endogenous growth hormone-releasing hormone (GHRH) (Guillemin *et al.* 1982, Rivier *et al.* 1982), attention has been focused upon the development of small peptides that possess similar growth hormone (GH)-releasing abilities (Momany *et al.* 1981, 1984). His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 (GHRP-6) was the first of these small peptides found to specifically release GH *in vitro* (Bowers *et al.* 1980, Badger *et al.* 1984, Sartor *et al.* 1985*a*) and *in vivo* in a variety of species (Sartor *et al.* 1985*b*, Ilson *et al.* 1989, Walker *et al.* 1990, Malozowski *et al.* 1991, Hartman *et al.* 1992). More recently, our group has reported the bioactivity of a new GHRP, His-D-2methyl-Trp-Ala-D-Phe-Lys-NH_2 (hexarelin) which is biologically active when given i.v., s.c. or orally in conscious adult rats (Conley *et al.* 1994, 1995) and which may be a more potent GH secretagogue than GHRP-6 (Conley *et al.* 1995).

  Printed in Great Britain
0022–0795/98/0158–0367 $08.00/0

The current study was precipitated by the fact that the clinical potential of any GHRP will not be realized until its effectiveness in eliciting GH release is fully characterized and until the mechanism by which it elicits GH release is understood. Therefore we investigated the effectiveness of hexarelin in eliciting GH release when administered as repeated i.v. doses at 2 h intervals or as continuous i.v. 6, 30 and 174 h infusions.

Materials and Methods

Animals

Adult male Sprague–Dawley rats (Sasco Inc., Madison, WI, USA) were used in this study. All animals were acquired, maintained and handled according to the guidelines established by the National Institutes of Health and as approved by the Animal Care and Use Committee at the University of Wisconsin-Milwaukee. After surgery, all animals were housed in a temperature- and humidity-controlled environment in individual isolation boxes under a 14 h light, 10 h darkness schedule (lights on at 0600 h). Food and water were available *ad libitum*.

Experimental preparation and procedures

Repeated doses experiment Eighteen adult male rats were prepared 3 days before experimentation with single indwelling jugular catheters under ether anesthesia (Obonsawin *et al.* 1985). Catheter construction and catheterization procedures have been detailed elsewhere (Wehrenberg *et al.* 1983, 1984). At 0730 h on the day of experimentation, all lines were outfitted with extensions and each animal received an i.v. bolus injection of heparin (100 IU). After 30 min, a baseline blood sample was drawn and each rat received a 25 μg/kg i.v. injection of hexarelin. This dose was chosen as we have previously shown it to consistently elicit GH release in the freely moving rat (Conley *et al.* 1994, 1995). Blood samples (0·2 ml) were collected at 5, 10, 15, 30, 60, 90 and 120 min after injection. The hexarelin treatment and the same blood sampling regimen were repeated at 120 and 240 min after the first hexarelin injection. Hematocrits were maintained by the periodic reinjection of resuspended red blood cells. All samples were immediately centrifuged and the plasma assayed for GH.

Continuous infusion experiment Rats were prepared with dual indwelling jugular catheters under either sodium pentobarbital or ether anesthesia. To keep the sampling catheters open during the recovery periods (7 and 3 days after pentobarbital and ether anesthesia respectively (Martin 1973, Obonsawin *et al.* 1985)) for the 6 and 30 h infusions, the infusion catheters were plugged and the blood sampling catheters were connected to swivels through which 2% heparinized saline was infused at a rate of 0·08 ml/h. (For the 174 h experiment, the sampling catheter was used for the treatment infusions which were started on the same day as surgery.) At the appropriate time before blood sampling, the infusion pumps for all groups were set to deliver fluid at a rate of 1 ml/h, which for the hexarelin-infused rats corresponded to 100 μg hexarelin/h. This dose and rate of infusion were chosen on the basis of our previous i.v. dose–response work with hexarelin (Conley *et al.* 1994), previously published reports on GHRP-6 infusion (Clark *et al.* 1989), and the extensive experience of one of us (W B W) with the continuous infusion of GH secretagogues (Wehrenberg 1986, Wehrenberg *et al.* 1986). On the day scheduled for blood withdrawal, the infusion catheters were connected via the swivels to the pumps and the blood sampling catheters were prepared with extensions. Each rat was then given a 100 IU bolus injection of heparin. In all cases, 0·2 ml blood samples were drawn every 20 min for a total of 6 h, with sampling starting between 0830 h and 0930 h (Wehrenberg 1986, Painson & Tannenbaum 1991, Butkus *et al.* 1995). Therefore, for the 6 h saline or hexarelin infusions, blood samples were collected over the duration of the infusion, the pumps having been started after a baseline blood sample. In the case of the 30 h infusion, the treatment infusions were started after an appropriate recovery period and the 6 h of blood sampling commenced 24 h later. Thus blood sampling occurred during hours 24 to 30 of the continuous infusions of either saline or hexarelin. For the 174 h infusion study, the saline or hexarelin infusions were started on the same day as surgery with blood samples collected during hours 168 to 174 of the infusions. All infusion pumps were stopped after the 6 h blood sample. Hemodilution was prevented by the periodic (after every 5th or 6th sample) reinjection of resuspended red blood cells. Blood samples were centrifuged immediately and the plasma frozen (− 20 °C) until assayed for GH, insulin-like growth factor-I (IGF-I) and corticosterone (RIA). In all cases, animals were observed for well-being and their infusion lines checked at least twice daily.

Peptide

Hexarelin was produced by conventional solid phase synthesis and was a gift from R Deghenghi (Europeptides Inc., Argenteuil, France). It was received in lyophilized form and reconstituted in normal saline and stored frozen (− 20 °C) in 1 mg/ml aliquots. Before experimentation, the stock aliquots were thawed and the peptide diluted with normal saline to achieve a working concentration of 100 μg/ml.

Radioimmunoassay

GH concentrations were determined in duplicate by RIA using a double-antibody method. Blood samples were

assayed in 10 μl aliquots. GH concentrations are expressed in terms of the NIH-RP-2 standard. The minimum sensitivity of the assay approximated 0·04 ng/tube and the maximum sensitivity was approximately 1·00 ng/tube. Within- and between-assay variability averaged less than 10%. After GH assay, the remaining plasma from the last hour of blood sampling was pooled for each animal in order to provide enough sample for IGF-I and corticosterone assay. IGF-I concentrations were determined by RIA after acid/ethanol extraction using a double-antibody method as previously described for humans (Miell *et al.* 1991), but with the substitution of a rat IGF-I primary antibody for the human IGF-I antibody. Blood samples (25 μl) were extracted and assayed in duplicate. The minimum sensitivity of the assay approximated 50 pg/tube and the maximum 2500 μg/tube. Within- and between-assay variability averaged less than 10%. Corticosterone concentrations were determined in duplicate using a single antibody/charcoal/dextran method (Spinedi *et al.* 1991). Blood samples were extracted using dichloromethane. The assay volume was 25 μl. The intra- and inter-assay coefficients of variation ranged from between 4 and 7, and 8 and 10% respectively. RIA reagents for these assays were provided by the National Pituitary Agency of the National Institutes of Health.

Data analysis

Repeated doses experiment For the repeated doses experiment, the mean peak GH response and the mean area under the GH curve (AUC) for the 30 min after each administration were calculated and are reported as the mean ± S.E.M. Significant differences in responses were identified using ANOVA with correction for repeated measures (Winer 1971). Individual differences were identified using the Studentized Range Statistic, *Q* (Zar 1974).

Continuous infusion experiment For the continuous infusion experiments, the mean GH AUCs for the entire 6 h blood sampling period were calculated and are reported as the mean ± S.E.M. for each treatment. Significant differences ($P<0·05$) between treatment groups were identified using one-way ANOVA. One-way ANOVA was also used to analyze possible differences between treatment groups for the corticosterone and IGF-I results. If main treatment effects were found by ANOVA, differences between treatment groups were identified using the Student–Newman–Keuls multiple range test with significance set at $P<0·05$. Since ANOVA failed to identify any effects of saline infusion on the parameters monitored, the data from saline-treated rats were pooled. The pulsatile character of GH release in individual rats was analyzed using PULSAR, a computer-assisted algorithm designed for the study of pulsatile hormone release (Merriam & Wachter 1982). Parameters analyzed were plasma GH peak height and duration, the interval between successive GH pulses and the number of peaks that occurred during the 6 h of blood sampling. GH trough concentrations were also evaluated. A trough was defined as the lowest GH nadir occurring between two GH peaks identified by PULSAR. Differences between treatments ($P<0·05$) for each of the parameters listed above were tested using one-way ANOVA.

Results

Repeated doses experiment

Repeated 25 μg/kg i.v. hexarelin injections given at 2 h intervals consistently elicited GH release, with peaks noted within 15 min of injection (Fig. 1A). Analysis of the GH peak and AUC data (Fig. 1B and C respectively) revealed that the first hexarelin injection precipitated a GH response that was significantly larger ($P<0·05$) than that noted after the second injection of hexarelin, but not significantly different from that noted after the third. In addition, the GH response after the third hexarelin injection did not differ from that noted after the first or second.

Continuous infusion experiment

Figure 2 presents representative examples of the pattern of GH release noted in individual rats from each of the four treatment groups, and Fig. 3 summarizes the average GH release noted over time in each of the four groups. As was noted in the saline controls, pulsatile GH release was maintained even in the presence of a 6, 30 or 174 h infusion of hexarelin (Fig. 2). For the 6 h infusion, a large increase in plasma GH was noted by 40 min after the start of hexarelin infusion (Figs 2B and 3A). After this peak, however, the plasma GH concentrations fell rapidly to levels that did not appear to differ from those noted in the saline-infused controls. Indeed, analysis of the AUC data for each of the 6 h blood sampling periods revealed that the mean GH concentrations noted were not significantly different between any of the four treatment groups (Fig. 3B).

Various characteristics of the pulsatile GH release noted in individual rats infused with either saline or hexarelin were analyzed using PULSAR (Table 1) (Merriam & Wachter 1982). No significant differences were identified between any of the hexarelin-infused treatment groups and the saline controls for the parameters of GH peak height and duration, GH trough concentration, interpeak interval and the total number of GH peaks that occurred during the 6 h sampling period.

Figure 4A summarizes the results obtained from our inquiry into what effect the hexarelin infusions may have

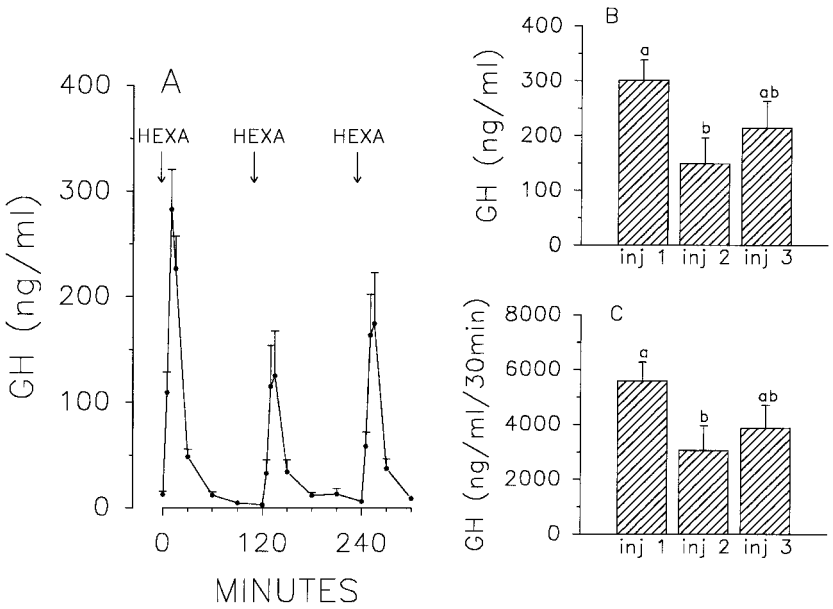

Figure 1 (A) The plasma GH responses noted in 18 conscious and freely moving adult male rats after three 25 μg/kg i.v. hexarelin (HEXA) injections given at 2 h intervals (arrows). Data points represent the mean ± S.E.M. for each time point. The two plots on the right summarize the GH peak (B) and area under the GH response curve (AUC) (C) for the first 30 min after each of the three i.v. 25 μg/kg hexarelin injections. The large GH response noted after the first injection was significantly higher than that noted in response to the second, although the GH response after the third hexarelin treatment did not differ from the first two. Significant differences ($P<0{\cdot}05$) are denoted by differing lower case letters. Data are expressed as the mean ± S.E.M. for both plots.

had on the plasma corticosterone concentrations noted during the last hour of each infusion. Here, although it appeared that the plasma corticosterone concentrations increased with the longer hexarelin infusions, no statistically significant differences were found between any of the treatment groups. In contrast, although no significant differences in plasma IGF-I concentrations were noted in response to the saline or to 6 and 30 h of hexarelin infusion, a 174 h hexarelin infusion did produce a significant elevation in plasma IGF-I concentrations ($P<0{\cdot}05$; Fig. 4B).

Discussion

We have previously reported that the GHRP hexarelin is able to elicit GH release in a dose-dependent fashion via a number of routes of administration in the conscious and freely moving rat (Conley *et al.* 1994) and have suggested that hexarelin may be a more potent GH secretagogue than GHRP-6 (Conley *et al.* 1995). In addition, we have also proposed that hexarelin, as well as the other GHRPs, may elicit GH release via a mechanism that suppresses functional somatostatin tone (Conley *et al.* 1995). In the current study, we examined the effects of hexarelin on GH release in conscious and freely moving adult male rats when given either as repeated i.v. doses (25 μg/kg) or as continuous i.v. infusions (100 μg/h) for total durations of 6, 30 or 174 h. These endeavors are a continuation of our previous investigations and were precipitated by the fact that the clinical potential of any GHRP will not be realized until its effectiveness in eliciting GH release is fully characterized and until the mechanism by which it precipitates GH release is understood.

Intravenous treatment of conscious adult male rats at 2 h intervals with 25 μg/kg hexarelin repeatedly elicited marked plasma GH responses (Fig. 1). Although there appeared to be a down-regulation in the plasma GH response to the second injection, subsequent recovery in the somatotroph responsiveness to hexarelin was suggested as the GH response elicited by the third hexarelin injection did not differ from that elicited by the first. The brief decrease in somatotroph responsiveness to these peptides is probably not due to depletion of GH stores, as numerous findings (including the GH responsiveness to subsequent hexarelin treatment just discussed) suggest ample pituitary reserves (Wehrenberg *et al.* 1983, Sartor *et al.* 1985*b*, Bowers *et al.* 1991). In addition, suppression of pituitary responsiveness to hexarelin in response to a coincident increase in somatostatin tone (during an endogenous GH

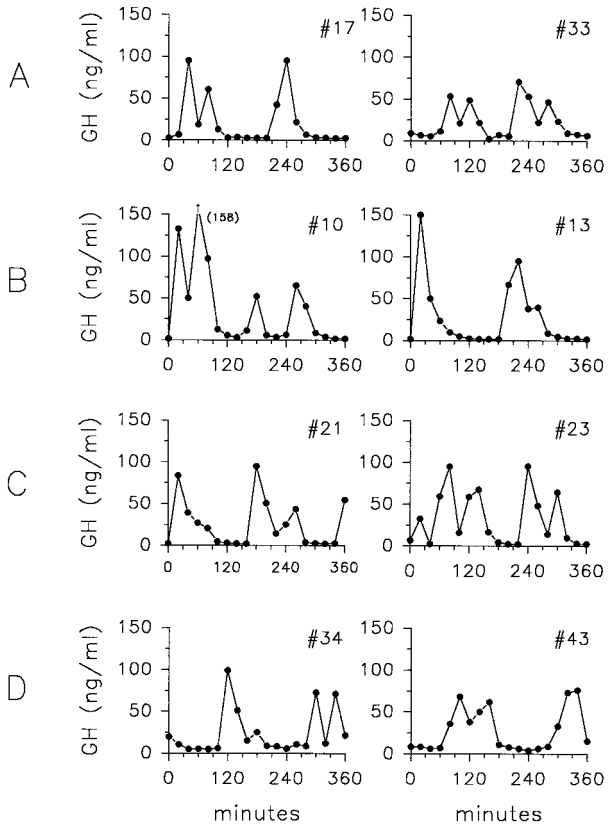

Figure 2 Representative examples of the GH responses noted in individual conscious and freely moving adult male rats infused with saline for 30 (#17) or 174 (#33) h (A), or hexarelin (100 μmg/h) for 6 (B), 30 (C) or 174 (D) h. Blood samples were drawn for the entirety of the 6 h infusions, while samples were taken during only the last 6 h of the 30 h and 174 h infusions. Characteristic male pattern GH release was maintained during all infusion regimens.

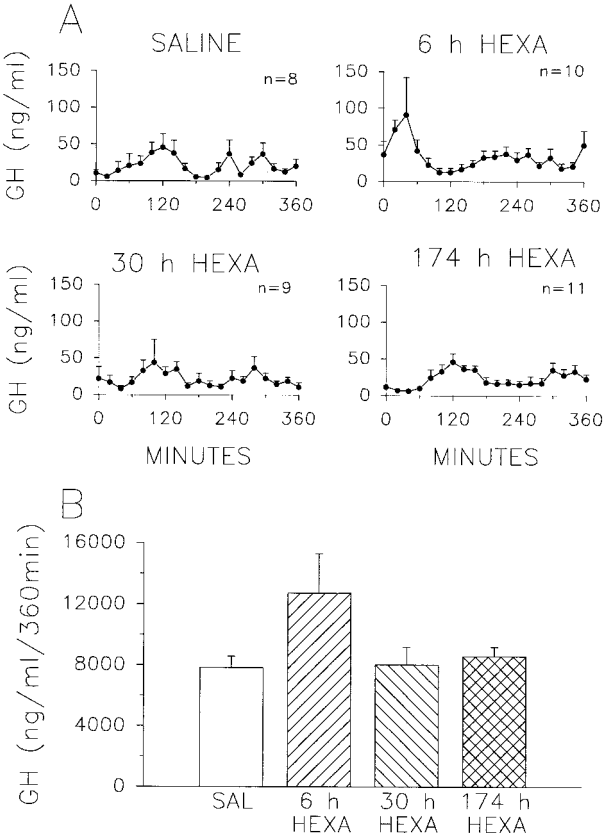

Figure 3 (A) The average GH responses noted after saline (1 ml/h) and 6, 30 or 174 h of hexarelin (HEXA) infusion (100 μg/h) in conscious and freely moving adult male rats. Blood samples were collected for the entire duration of the 6 h hexarelin infusion, but only for the last 6 h of the two longer hexarelin infusion periods. The plot for the saline group represents a pool of the data collected for the 6, 30 and 174 h saline infusions. In all cases, pulsatile GH release was maintained. A marked increase in plasma GH concentrations was noted within the first 40 min of hexarelin infusion initiation (6 h HEXA), after which plasma GH concentrations fell to levels that did not appear to differ from those noted in the saline-infused rats. Data points represent the mean ± S.E.M. for each time point. (B) Summary of the integrated AUCs for the 6 h of blood sampling in animals that received saline (n=8) or hexarelin (HEXA) for 6 h (n=10), 30 h (n=9) or 174 h (n=11). No significant differences in the integrated AUCs were found between any of the four treatment groups. Data presented are the mean ± S.E.M. for each group.

trough) is also an unlikely explanation, as we have previously shown that the GH-releasing capabilities of hexarelin do not differ during conditions of high versus low somatostatin tone (Conley *et al.* 1995). Thus it would appear that, since the GH axis remains responsive to repetitive hexarelin challenge with minimal down-regulation in response, this new GHRP holds the potential to be used chronically in the clinical setting to maintain enhanced GH activity.

Administration of hexarelin as a continuous i.v. infusion did not appear as effective as the intermittent treatments (as described in the current study and reported previously (Conley *et al.* 1994, 1995)) at eliciting GH release. This difference may reflect the fact that a 100 μg dose of hexarelin infused over 1 h produces a much smaller realized dose than does a 25 μg/kg dose administered over 10–20 s. Although an initial surge in GH release was noted in response to the start of the hexarelin infusion, plasma GH concentrations had returned to basal levels by 60 min (Figs 2 and 3). This return to basal secretion is reinforced by the fact that the average GH concentrations (6 h integrated AUCs) did not differ between any of the treatment groups (Fig. 3B). A similar pattern of GH release has been reported with continuous GHRP-6 exposure both *in vitro* (Blake & Smith 1991) and *in vivo* (Badger *et al.* 1984, Clark *et al.* 1989). Possible explanations for these observations could be a GHRP-induced depletion of pituitary GH stores, an increase in an endogenous factor that inhibits GH release with prolonged exposure

Table 1 Summary of the data obtained from PULSAR analysis of various characteristics of pulsatile GH release. Data are expressed as the mean ± S.E.M.

	Saline	Hexarelin		
		6 h	30 h	174 h
GH peak height (ng/ml)	79 ± 11	92 ± 19	70 ± 16	60 ± 4
GH troughs (ng/ml)	8 ± 2	16 ± 5	5 ± 1	13 ± 3
Peak duration (min)	31 ± 3	44 ± 5	31 ± 4	43 ± 5
Interpeak interval (min)	131 ± 17	118 ± 15	123 ± 15	118 ± 20
Number of peaks/6 h	2·9 ± 0·3	3·0 ± 0·5	3·1 ± 0·4	2·7 ± 0·2

to these GH secretogogues, or somatotroph receptor or post-receptor changes which produce a subsequent decreased responsiveness to hexarelin. A number of observations suggest that a change in receptor responsiveness is the most likely explanation (Bilezikjian & Vale 1984, Bilezikjian *et al.* 1986, Wehrenberg *et al.* 1986, Blake & Smith 1991). For example, a hexarelin-induced depletion of pituitary GH stores is not likely, as we have previously shown that rats continuously exposed for 6 h to i.v. hexarelin remain responsive to subsequent GHRH stimulation (L K Conley, R C Gaillard, A Giustina, R S Brogan & W B Wehrenberg, unpublished observations) and others have reported a maintained responsiveness to GHRH or the opiate agonist MRZ 2549 during continuous GHRP-6 exposure (Sartor *et al.* 1985*b*, Clark *et al.* 1989). In addition, although others have reported increases in cortisol in response to intermittent GHRP-6 treatment in man (Hayashi *et al.* 1991, Frieboes *et al.* 1995, Thomas *et al.* 1997), a sustained increase in this inhibitor of GH secretion (Wehrenberg *et al.* 1990) is an unlikely explanation, as we found that corticosterone concentrations were not significantly elevated in response to long-term exposure to hexarelin (Fig. 4A). Likewise, an increase in the primary inhibitor of GH release, somatostatin, is an unlikely determinant in this monophasic pattern of GH release as (1) we noted no change in the characteristic male pattern of GH release in the current study (Table 1) (Tannenbaum & Ling 1984, Plotsky & Vale 1985, Arsenijevic *et al.* 1987) and (2) the GH responsiveness to MRZ 2549, hypothesized to work via a hypothalamic mechanism quite possibly involving somatostatin, is not altered by GHRP-6 infusion (Sartor *et al.* 1985*b*).

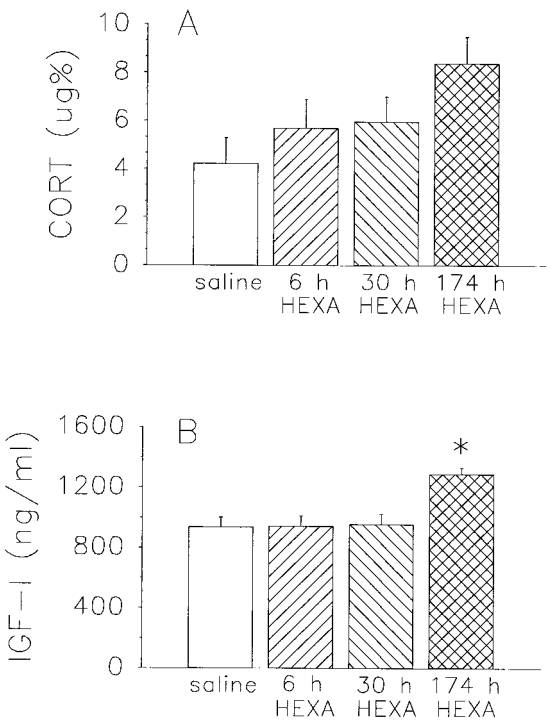

Figure 4 (A) The plasma corticosterone (CORT) concentrations noted during the last hour of either saline or each of the hexarelin (HEXA) infusions. No significant differences were noted between any of the treatment groups. (B) The plasma IGF-I concentrations noted during the last hour of either saline or each of the hexarelin (HEXA) infusions. IGF-I concentrations were significantly elevated above those noted in all other treatment groups in response to the 174 h hexarelin infusion (*P<0·05). Data are expressed as the mean ± S.E.M. for both plots.

Our observation that the hexarelin infusions did not alter any parameter of pulsatile GH release is also of note when considering possible mechanisms for GHRP-induced GH release. Interestingly, these findings are in contrast with a study that reported that the characteristic male pattern of pulsatile GH release was 'less apparent' and that 'GH levels' (presumably peak and trough concentrations) were enhanced in rats infused for 6 h with the same dose and rate of GHRP-6 (Clark *et al.* 1989). The differences between the results of that study and ours may not be so distinct since it is not readily apparent what methodology was used to analyze the character of pulsatile GH release in the other study and since different controls were used in the two studies. In this regard, the former study used 1·5 to 2 h of pre-infusion blood sampling to characterize the control response, while we used separate groups of saline-infused rats to do this. The alteration in GH pulse character and, in particular, the elevation of GH trough concentrations in response to a continuous infusion of GHRP-6 reported in the earlier study suggest some

modulation of functional somatostatin tone by the GHRPs (Wehrenberg *et al.* 1982, Tannenbaum & Ling 1984). Our observation that neither the timing and character of GH pulses nor the height of GH troughs are affected by long-term exposure to hexarelin, on the other hand, argues against this hypothesis. Clearly, further investigation of somatostatin involvement in the mechanism by which the GHRPs elicit GH release is warranted.

It is also of interest that, although no significant differences in the average plasma GH concentrations were noted between rats infused with saline or hexarelin for 6, 30 or 174 h (Fig. 3), a 174 h continuous hexarelin infusion resulted in a significant elevation in the average plasma IGF-I concentrations (Fig. 4B). While it is granted that many factors affect IGF-I synthesis and release, the fact remains that animals treated similarly to controls but for 174 h of hexarelin exposure (a duration long enough to be far removed from whatever stimulatory effects the initial surge in plasma GH might have had) exhibited significantly elevated concentrations of circulating IGF-I. The importance of this finding is at present unclear in relation to the prevailing GH tone and also warrants further investigation.

The current findings also provide support for the clinical promise of hexarelin and the other synthetic GH secretagogues. For example, the stimulation of glucocorticoid release by a GH secretagogue used clinically for treatment of GH deficiency is a concern, as these corticosteroids can inhibit the growth-promoting actions of the GH axis and have other troublesome side effects (Giustina & Wehrenberg 1992). Although activation of the hypothalamic–pituitary–adrenal axis has been reported after a single intravenous injection of GHRP-6 (Thomas *et al.* 1997), our observation that plasma corticosterone concentrations were not changed in response to 6, 30 or 174 h of continuous hexarelin exposure suggests that any increases in circulating glucocorticoids in response to GHRP treatment may be an early acute effect. The short-lived nature of hexarelin-induced glucocorticoid release is supported by a report that long-term intranasal hexarelin administration does not elicit significant increases in cortisol levels in children (Laron *et al.* 1995). In addition, 7 to 14 days of daily MK-677 oral treatments failed to significantly increase serum cortisol concentrations in normal young men or in elderly men and women (Copinschi *et al.* 1996, Chapman *et al.* 1996). The potential clinical usefulness of these synthetic GH secretagogues is also reinforced by observations that long-term treatment produces elevations in circulating IGF-I concentrations. For example, significant increases in plasma IGF-I concentrations were noted in the current study after 7 days of hexarelin infusion, and have been reported in prepubertal children after 7 days of intranasal hexarelin administration (Laron *et al.* 1995) and in adults after 7 to 14 days of oral MK-677 treatment (Copinschi *et al.* 1996). And finally, it is of interest that long-term intermittent hexarelin treatment has been shown to significantly increase circulating GH concentrations in both children of short stature (Laron *et al.* 1995, Klinger *et al.* 1996) and in aged dogs (along with an improvement in some indices of body composition) (Cella *et al.* 1996). Thus, the potential clinical utility of these GH secretogogues in the treatment of GH deficiency is reinforced by their ability to stimulate GH and IGF-I release after prolonged treatment, without a concomitant activation of the hypothalamic–pituitary–adrenal axis. Furthermore, hexarelin and the other synthetic GH secretagogues appear to be able to elicit these effects in individuals of many ages.

In conclusion, the results of the present study demonstrate that hexarelin is capable of modulating the function of the growth axis when given as either intermittent i.v. injections or as continuous i.v. infusions of varying lengths. In addition, we have provided evidence that the mechanism by which hexarelin and the other GHRPs elicit GH release most likely does not involve suppression of functional somatostatin tone. Our findings collectively illustrate the complex nature by which these synthetic peptides elicit GH release and emphasize that further description of their physiological effects and continued investigation into their mechanism of action is warranted.

Acknowledgements

The authors thank J Dargatz and L Yee for their help in the preparation of the manuscript and M Giacomici, I Heimler, L Stagg and E Temler for their technical assistance on this project. We also express our gratitude to the NIDDK division of the National Institutes of Health for the generous supply of RIA reagents and R Deghenghi (Europeptides Inc., Argenteuil, France) for providing hexarelin. This work was funded by Mediolanum Pharmaceutici (Milano, Italy). The participation of L K C in this study was funded, in part, by the Graduate School of the University of Wisconsin-Milwaukee.

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Received 4 June 1997
Revised manuscript received 20 February 1998
Accepted 20 April 1998