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Acute administration of hexarelin stimulates GH secretion during day and night in normal men

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Summary

OBJECTIVE Hexarelin is a synthetic hexapeptide with potent GH-releasing activity in both animals and men. The aim of this study was to investigate the effect of a bolus injection of hexarelin given in the morning during wakefulness and during nocturnal sleep in a group of normal adult men.

DESIGN AND SUBJECTS Eight normal men, aged 21–33 years, of normal height and within 10% of ideal body weight were studied. All subjects received in random order saline or hexarelin (2 μg/kg) in the morning between 0800 and 0900 h after they had fasted overnight. The same experiments were performed during nocturnal sleep in the same subjects. Saline or hexarelin were injected within 30 minutes after the onset of sleep between 2300 and 2400 h. Sleep was recorded by visual inspection.

MEASUREMENTS In all four test sessions blood samples were taken 30, 15 minutes and immediately before the injection of saline or hexarelin and then every 15 minutes for 2 hours. GH was measured by an immunoradiometric assay. All values are expressed as peak GH levels or as area under the curve (AUC) calculated by trapezoidal integration.

RESULTS Mean peak GH concentrations after hexarelin during the morning (58·2 $\pm$ 4·7 μg/l) (GH μg/l x 2 = mU/l) were not different from those observed during sleep (61·2 $\pm$ 4·3 μg/l). The rate of disappearance of GH from plasma was slower during sleep ($t_{1/2}$ = 64·9 $\pm$ 14·8 min) than during morning hours ($t_{1/2}$ = 24.9 $\pm$ 1·4 min, $P < 0·01$). Mean AUC responses to hexarelin during sleep (1466 $\pm$ 145 μg.min/l) were significantly higher than during morning hours (903 $\pm$ 94 μg.min/l, $P < 0·001$).

CONCLUSIONS These results show that GH responsiveness to a growth hormone releasing peptide is preserved during the night. This could be exploited for diagnostic and/or therapeutic purposes.

A series of small GH-releasing peptides (GHRPs) that stimulate GH secretion has been recently synthesized (Bowers, 1993). *In vitro*, GHRPs stimulate GH secretion from pituitary cells by a mechanism not mediated by either GHRH or opioid receptors (Cheng *et al.*, 1989; 1991; Codd *et al.*, 1989; Sethumadhavan *et al.*, 1991; Blake & Smith, 1991; Goth *et al.*, 1992), and via signalling mechanism distinct from those of GHRH (Cheng *et al.*, 1989; 1991). However, recent data indicate that the hypothalamus is the principal site of action of GHRPs. This view is supported by the findings that the GH-releasing activity of GHRPs *in vitro* is far less potent than *in vivo* or when the *in vitro* experiments are carried out on hypothalamic-pituitary incubates (Bowers *et al.*, 1991), and by the evidence of specific hypothalamic binding sites for the peptide (Codd *et al.*, 1989; Sethumadhavan *et al.*, 1991). In addition, the GH responses to GHRPs are absent or markedly blunted both in animals (Fletcher *et al.*, 1994) and in humans (Popovic *et al.*, 1995; Loche *et al.*, 1995a) with hypothalamopituitary disconnection. The intracerebroventricular administration of GHRPs in rats elicits a large GH response at very low doses which are ineffective after i.v. administration (Fairhall *et al.*, 1995). Furthermore, GHRPs act synergistically with GHRH to release GH both *in vitro* (Cheng *et al.*, 1989; 1991; Bowers *et al.*, 1991) and *in vivo* (Bowers *et al.*, 1990; Popovic *et al.*, 1995).

It is well known that GH is secreted in a pulsatile manner throughout the day, with an amplification of GH peaks during the first hours of nocturnal sleep. Data obtained from pharmacological studies indicate that the sleep-induced GH secretion may be associated with a decreased somatostatin (SRIH) tone. In fact, nocturnal GH secretion is enhanced during continuous GHRH infusion (Vance *et al.*, 1985), and the response to a GHRH bolus is greater during sleep than during

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waking hours (Van Cauter *et al.*, 1992a). Furthermore, nocturnal GH secretion is not enhanced by administration of clonidine (Loche *et al.*, 1989; Ghigo *et al.*, 1990a), pyridostigmine (Ghigo *et al.*, 1990b), or atenolol (Martha *et al.*, 1988), all drugs whose GH-releasing activity is likely to be mediated by inhibition of endogenous SRIH release. However, it has been recently reported that infusion of a GHRH antagonist eliminates nocturnal GH secretion in man, thus suggesting a pivotal role for endogenous GHRH in the generation of nocturnal GH pulsatility (Jaffe *et al.*, 1993a). Enhancement of nocturnal GH secretion has been observed during continuous infusion of GHRP-6 in normal man (Huhn *et al.*, 1993; Jaffe *et al.*, 1993b).

Hexarelin is a synthetic hexapeptide (His-D-2-methyl-Trp-Ala-Trp-D-Phe-Lys-NH_2) similar to GHRP-6, in which D-tryptophan has been replaced by its 2-methyl derivative (Conley *et al.*, 1994). Recent studies have shown that hexarelin is a potent GH secretagogue after intravenous, intranasal or subcutaneous administration both in adults (Ghigo *et al.*, 1994; Imbimbo *et al.*, 1994) and children (Laron *et al.*, 1994; Loche *et al.*, 1995a,b; Bellone *et al.*, 1995).

In this study we have investigated the effect of a bolus injection of hexarelin given in the morning during wakefulness and during nocturnal sleep in a group of normal adult men.

Subjects and methods

Eight normal men, aged 21–33 years, of normal height and within 10% of ideal body weight, were studied after they had given informed consent. None had any medical illness or took medications. The study was carried out under institutionally approved protocols. All subjects were studied on four occasions, in a random order, and tests were performed with an interval of 7–15 days. The morning studies started between 0800 and 0900 h after the subjects had fasted overnight. An intravenous forearm cannula was inserted and blood samples were taken 30, 15 minutes and immediately before a bolus injection of saline or hexarelin (prepared and supplied by Europeptides, Argenteuil, France) at the dose of 2 μg/kg. Blood was then sampled every 15 minutes for 2 hours. Subjects were confined to bed and remained awake during the entire test sessions. The same experiments were performed during nocturnal sleep in the same subjects. An intravenous forearm cannula was inserted between 2100 and 2200 h and lights were off at 2300 hours. Sleep was carefully recorded by visual inspection. Saline or hexarelin were injected within 30 minutes after the onset of sleep between 2300 and 2400 h, and blood samples were collected as in the morning studies. Care was taken not to disturb sleep, and none of the subjects woke during the experiments.

GH was measured by an immunoradiometric assay (HGH-CTK-IRMA, Sorin, Italy). The sensitivity of the assay was 0·2 μg/l (0·4 mU/l) with intra and inter-assay coefficients of variation of 4·5 and 7·9%, respectively. The area under the curve (AUC) was calculated by trapezoidal integration. The rate of disappearance of GH from plasma was estimated with log-linear regression of the post-absorption mean concentrations. The slopes of the regression lines represent the elimination rate constants (K_e) from plasma. The corresponding elimination half-lives ($t_{1/2}$) were defined as In (2)/K_e (Imbimbo *et al.*, 1994). Statistical analysis of the results were performed using the paired *t*-test. All values are given as the mean ± SEM.

Table 1 Individual GH peak (μg/l) and AUC (μg.min/l) responses to hexarelin (2 μg/kg i.v.) administered in the morning and during nocturnal sleep in 8 normal adult men. *$P < 0·001$ *vs* morning AUC. GH μg/l × 2 = mU/l.

	Morning		Night	
Case no.	Peak	AUC	Peak	AUC
1	48·8	740	60·8	1022
2	52·5	903	55·4	1660
3	37·8	491	46·2	958
4	62·4	1401	63·2	2101
5	64·3	1070	50·8	1151
6	49·5	758	55·3	1352
7	69·5	938	71·2	1638
8	77·3	921	83·5	1843
Mean±	58·2	903	61·2	1466*
SEM	4·7	94	4·3	145

Results

Hexarelin administration was well tolerated and did not induce relevant side-effects in any of the subjects. In 7 subjects baseline GH concentrations (mean of 3 determinations) before hexarelin injection in the morning experiments were <1·0 μg/l (GH μg/l × 2 = mU/l), while in the remaining subject (case 4 of Table 1) baseline GH concentrations were 5·6 μg/l. Baseline GH concentrations before hexarelin injection in the night experiments were <1·0 in 6 subjects, and were 2·0 and 3·0 μg/l in subjects 1 and 4, respectively. As a mean baseline GH concentrations before hexarelin injection were similar between the morning (1·2 ± 0·6 μg/l) and the nocturnal (1·1 ± 0·3 μg/l) experiments. During the placebo experiments, mean peak GH concentrations (9·4 ± 1·8 μg/l) and mean AUC (123 ± 28·3 μg.min/l) during night hours were significantly higher than during morning hours (peak 2·1 ± 0·8 μg/l, $P < 0·02$; AUC 35·6 ± 14·5 μgmin/l, $P < 0·05$) (Fig. 1). Individual GH peak and AUC responses to hexarelin during the morning and during sleep are shown in Table 1. The maximum GH peak following hexarelin administration occurred between 15 and 30 minutes

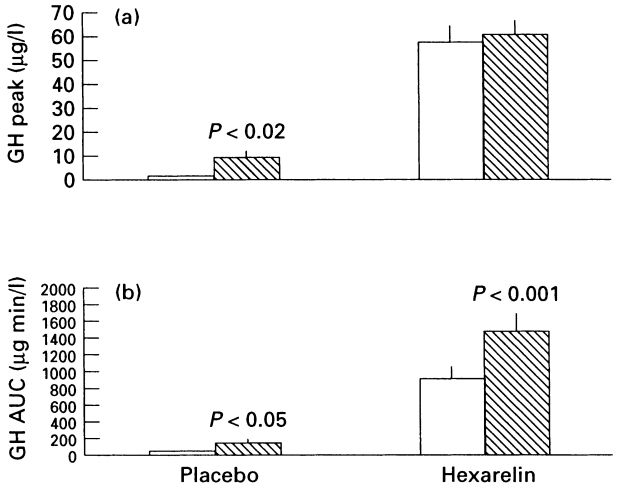

Fig. 1 Mean (±SEM) a, GH peak; b, AUC responses to placebo and to hexarelin (2 μg/kg i.v.) during □, morning hours and ▨, nocturnal sleep in 8 normal men. GH μg/l × 2 = mU/l.

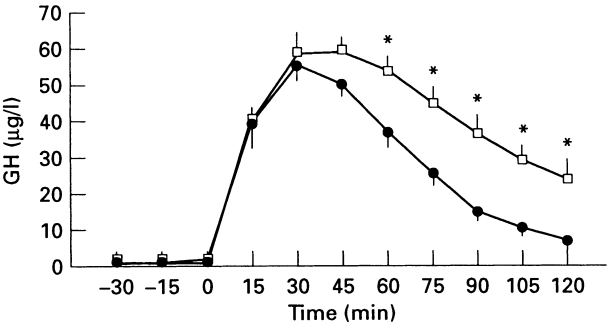

Fig. 2 Mean (±SEM) serum GH responses to i.v. bolus injection of hexarelin (2 μg/kg) during ●, morning hours; □, nocturnal sleep in 8 normal adult men. * Significantly different *vs* corresponding time values of the morning experiments. GH μg/l × 2 = mU/l

in both the morning and the night experiments. Mean peak GH concentrations after hexarelin during the morning (58·2 ± 4·7 μg/l) were not different from those observed during sleep (61·2 ± 4·3 μg/l) (Fig. 1). The rate of disappearance of GH from plasma was slower during sleep ($t_{1/2}$ = 64·9 ± 14·8 min) than during morning hours ($t_{1/2}$ = 24·9 ± 1·4 min, $P < 0·01$). Mean GH concentrations at time 60 (37·5 ± 3·9 *vs* 54·0 ± 4·6 μg/l, $P < 0·01$), 75 (26·4 ± 4·1 *vs* 44·7 ± 4·4 μg/l, $P < 0·01$), 90 (15·5 ± 3·0 *vs* 37·2 ± 5·0 μg/l, $P < 0·002$), 105 (10·9 ± 1·8 *vs* 29·6 ± 4·6, $P < 0·002$) and 120 min (7·3 ± 1·5 *vs* 24·3 ± 5·2 μg/l, $P < 0·01$) were significantly higher during sleep than during the morning hours (Fig. 2). As a result of the slower clearance, mean AUC responses to hexarelin during sleep (1466 ± 145 μg.min/l) were significantly higher than during morning hours (903 ± 94 μg.min/l, $P < 0·001$) (Fig. 1).

Discussion

This study confirms that hexarelin is a potent GH-releasing stimulus in man (Ghigo *et al.*, 1994; Imbimbo *et al.*, 1994; Laron *et al.*, 1994; Arvat *et al.*, 1995; Bellone *et al.*, 1995; Loche *et al.*, 1995a,b). In addition, we have shown that the potent GH-releasing activity of hexarelin is preserved during the first hours of nocturnal sleep. Our findings are in agreement with the observation that continuous infusion of GHRP-6 enhances nocturnal GH secretion in normal man (Huhn *et al.*, 1993; Jaffe *et al.*, 1993b). However, Jaffe *et al.* (1993b) found that GHRP-6 was least effective in augmenting GH secretion during the period corresponding to nocturnal augmentation of GH release, while we found that the GH response to an i.v. bolus of hexarelin during nocturnal sleep was similar to that observed during morning hours. These discrepancies may be due to the different experimental conditions, i.e. continuous i.v. infusion *vs* bolus injection. Evaluation of the rate of disappearance of GH from plasma showed that the elimination $t_{1/2}$ of the hormone was significantly increased at night. This difference in GH clearance probably accounted for the higher AUC observed during the night experiments. The difference in the AUC between morning and night placebo experiments cannot account for the difference observed following hexarelin administration. In fact, the mean AUC during the night hexarelin experiments were significantly higher than the sum of the AUC of the day hexarelin and the night placebo experiments.

The hypothalamus is the principal site of action of the GHRPs (Fletcher *et al.*, 1994; Popovic *et al.*, 1995; Loche *et al.*, 1995a; Fairhall *et al.*, 1995), although their mechanisms of action are not completely understood. Data in experimental animals indicate that the effect of GHRPs on GH secretion is mediated by endogenous GHRH. Administration of anti-GHRH serum inhibits the GH response to GHRP-6 in rats (Clark *et al.*, 1989; Bowers *et al.*, 1991). In addition, increased release of GHRH without changes in SRIH secretion has been observed in sheep (Guillaume *et al.*, 1994) and rats (Conley *et al.*, 1995) after administration of hexarelin. On the other hand, studies in man also support the concept of a SRIH-mediated mechanism of action. In fact, GHRPs act synergistically with GHRH to release GH (Bowers *et al.*, 1990; Popovic *et al.*, 1995), and the GH response to GHRP-6 is maintained during continuous infusion of GHRH (Robinson *et al.*, 1995). Furthermore, the GH-releasing effect of hexarelin is not influenced by pretreatment with drugs, such as arginine or pyridostigmine, known to inhibit SRIH release (Arvat *et al.*, 1995). It has also been suggested that GHRPs may stimulate the release of an unidentified hypothalamic factor (Bowers *et al.*, 1991).

Nocturnal GH secretion is probably due to an intrinsic circadian rhythmicity as well as to the effects of sleep (Ghigo *et al.*, 1990b; Van Cauter *et al.*, 1992a,b). The time of maximal propensity for GH secretion, i.e. the first hours of nocturnal sleep, seems to be associated with a reduced SRIH tone (Vance *et al.*, 1985; Martha *et al.*, 1988; Loche *et al.*, 1989; Ghigo *et al.*, 1990a,b; Van Cauter *et al.*, 1992a,b). However, recent findings indicate that GHRH is essential for night-time GH secretion, as shown by the ability of a GHRH antagonist to abolish nocturnal GH pulsatility (Jaffe *et al.*, 1993a). Since GHRPs and GHRH are synergistic both *in vitro* (Cheng *et al.*, 1989; 1991; Bowers *et al.*, 1991) and *in vivo* (Bowers *et al.*, 1990; Popovic *et al.*, 1995), it may be speculated that in our experiments hexarelin potentiated a nocturnal surge of GHRH, independent of SRIH secretion and/or action.

Whatever may be the exact mechanisms by which hexarelin stimulates GH secretion, we have shown in this study that a single dose of hexarelin administered within 30 minutes from the onset of sleep markedly stimulates GH secretion. Our findings are of potential clinical relevance. In children most of the total GH secreted is produced at night. Therefore, to mimic physiological secretion, GH is best administered during the late evening hours in GH-deficient children. The potent GH-releasing effect of GHRPs or the recently developed non-peptide GHRP agonist (Smith *et al.*, 1993; Gertz *et al.*, 1993) and their effectiveness after oral (Bowers *et al.*, 1992; Ghigo *et al.*, 1994; Bellone *et al.*, 1995) and intranasal administration (Ghigo *et al.*, 1994; Laron *et al.*, 1994; 1995) make this family of peptides potentially useful therapeutic agents (Laron *et al.*, 1995; Mericq *et al.*, 1995).

In conclusion, we have shown that GH responsiveness to GHRPs is preserved during the night. This could be exploited for diagnostic and/or therapeutic purposes.

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