The GH, prolactin, ACTH and cortisol responses to Hexarelin, a synthetic hexapeptide, undergo different age-related variations

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Abstract
Hexarelin (HEX) is a synthetic growth hormone-releasing peptide (GHRP) which acts on specific receptors at both the pituitary and the hypothalamic level to stimulate GH release in an age-dependent manner. Like other GHRPs, HEX possesses also prolactin (PRL) and ACTH/cortisol-releasing activity, similar to that of human corticotropin-releasing hormone (hCRH). The mechanisms underlying the stimulatory effect of GHRPs on lactotrope and corticotrope secretion are even less clear and the influence of age on these endocrine activities of GHRPs is unknown. To clarify this point we studied the GH, PRL, ACTH and cortisol responses to the maximal effective dose of HEX (2.0 mg/kg i.v.) in: 12 prepubertal children (Pre-C, 8 male, 4 female, age 5.8–12.1 years); 12 pubertal normal short children (Pub-C, 5 male, 7 female, age 9.7–15.5 years, pubertal stage II–IV); 20 normal young adults (Young, 6 males, 14 females, age 23–32 years); and in 16 normal elderly people (Elderly, 5 male, 11 female, age 66–81 years). The GH response to HEX was clear in Pre-C (0–120 min area under curve, mean ± S.E.M. 769.5 ± 122.2 mg*min/l) but strikingly increased (P<0.001) in Pub-C (1960.2 ± 283.5 mg*min/l). The HEX-induced GH rise in Young (1829.7 ± 243.1 mg*min/l) persisted similar to that in Pub-C, but decreased in Elderly (951.1 ± 232.9 mg*min/l, P<0.005); the latter was, in turn, similar to that in Pre-C. HEX induced a significant PRL increase which, however, showed no age-related variations, being similar in Pre-C (512.1 ± 88.0 mg*min/l), Pub-C (584.0 ± 106.0 mg*min/l), Young (554.9 ± 56.0 mg*min/l) and Elderly (523.9 ± 59.6 mg*min/l). The ACTH-releasing activity of HEX was present in Pre-C (1356.6 ± 204.9 pg*min/ml) and was clearly enhanced (P<0.02) in Pub-C (2253.5 ± 242.8 pg*min/ml). The ACTH rise after HEX in Young (1258.1 ± 141.2 pg*min/ml) was lower (P<0.02) than that in Pub-C and similar to that in Pre-C, while the ACTH response to HEX in Elderly (1786.5 ± 340.1 pg*min/ml) showed a further trend toward increase, being similar to that in Pub-C. On the other hand, the cortisol response to HEX showed no significant age-related variations, being not different in Pre-C (7477.2 ± 1031.6 µg*min/l), Pub-C (6106.0 ± 862.9 µg*min/l), Young (6827.5 ± 509.6 µg*min/l) and Elderly (7950.6 ± 658.3 µg*min/l). In conclusion, our present data demonstrate that in humans the GH- and ACTH-releasing activities of HEX undergo different age-related variations, while its PRL-releasing activity is not dependent on age. These findings suggest that actions at different levels and/or on different receptor subtypes mediate the different age-related hormonal effects of GHRPs.

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Introduction
Growth hormone-releasing peptides (GHRPs) and non-peptidyl GHRP mimetics are synthetic, non-natural molecules with potent stimulatory effects on somatotrope secretion both in animals and in humans, being active even after oral administration (1–3). The GHRP activity, however, is not fully specific for growth hormone (GH) secretion. In fact, they possess also a stimulatory effect on prolactin (PRL), adrenocorticotropic hormone (ACTH) and cortisol secretion (4–9). Noteworthy, the PRL response to GHRPs is really slight and markedly lower than that to thyrotropin-releasing hormone (TRH), while the ACTH- and cortisol-releasing activity of GHRPs is similar to that of human corticotropin-releasing hormone (hCRH) (9).

Though synthetic and non-natural, GHRPs bind to specific receptors at the pituitary level as well as within the central nervous system (CNS), particularly in the hypothalamus, though GHRP binding at the hippocampal and the cortical level is remarkable (10–12). The GHRP receptor has recently been cloned and shows no significant homology with any other G-protein coupled receptor known so far (13).
The mechanisms underlying the GH-releasing activity of GHRPs are not definitively clarified. It has been hypothesized that they act concomitantly at the pituitary and the hypothalamic level (14), probably antagonizing somatostatinergic activity and facilitating the activity of GHRH-secreting neurons (15–17). The evidence that the GH response to GHRPs is strongly reduced, though not lost, in the presence of hypothalamo–pituitary disconnection (14, 18–20) suggests that the functional integrity of the hypothalamo–pituitary unit is needed for the full GH-releasing activity of GHRPs, and that the hypothalamic action has a major role.

The mechanisms underlying the stimulatory effect of GHRPs on ACTH and cortisol secretion have been even less studied. Data obtained so far indicate that the stimulatory effect on cortisol levels is due to an ACTH-releasing effect of GHRPs, which probably depends on CNS-mediated actions. In animals the ACTH and cortisol response to GHRPs is completely lost in the presence of hypothalamo–pituitary disconnection (20). Animal data suggest that GHRPs could act via a CRH-mediated action (21). In humans, the ACTH response to GHRPs is not modified by naloxone, an opioid antagonist (7), nor by cyproheptadine, a serotoninergic antagonist, or diphenhydramine, an histaminergic antagonist (22); this suggests that the ACTH-releasing activity of GHRPs does not depend on mediation by opioids, serotonin and histamine, which are known to play a major role in the control of the hypothalamo–pituitary–adrenal (HPA) axis (23).

Concerning the PRL-releasing activity of GHRPs, very little is known about the mechanism underlying this effect, though a direct effect at the pituitary level has been hypothesized (24, 25).

The endocrine activities undergo important age-related variations. In humans, this has been clearly shown for the GH/insulin-like growth factor-I (IGF-I) axis, whose function undergoes an increase at puberty and decreases thereafter up to very low activity in aging (3, 26, 27). The age-related variations of lactotrope and corticotrope secretions are less clear, though there are data pointing to an increase in aging (28, 29). There is evidence indicating that age-related changes in the secretion of anterohypophyseal hormones reflect age-dependent variations in the neural control of the anterior pituitary (3, 26–29), though an important influence is also played by gonadal steroids (30–32). The GH-releasing activity of GHRPs undergoes age-related variations partially different from those of GHRH (3, 33). However, it is still unknown whether the PRL- and the ACTH-releasing effects of GHRPs also vary across the human lifespan and whether or not they are associated with those of the GH-releasing activity.

To clarify this point, we studied the GH, PRL, ACTH and cortisol responses to Hexarelin (HEX), a synthetic hexapeptide belonging to the GHRP family (34), in both prepubertal children (Pre-C) and pubertal children (Pub-C), in young adults (Young) and in elderly subjects (Elderly).

**Subjects and methods**

**Peptides and drugs**

Vials containing 100 µg lyophilized HEX were kindly provided by Europeptides (Argenteuil, France).

**Study design**

Twelve Pre-C (8 male, 4 female, age 5.8–12.1 years), 12 Pub-C normal short children (5 male, 7 female, age 9.7–15.5 years, pubertal stage II–IV), 20 normal Young (6 male, 14 female, age 23–32 years) and 16 normal Elderly (5 male, 11 female, age 66–81 years) were studied. All subjects were within 15% of their ideal body weight. All were in good health and none was taking any medication known to influence GH, PRL or ACTH secretion. The pubertal girls and young women were studied in the follicular phase of their menstrual cycles.

The study had been approved by the local Ethical Committee and informed consent was obtained from all subjects, children and their parents.

All subjects underwent HEX treatment (2.0 µg/kg i.v. at 0 min). The test started between 0830 h and 0900 h after an overnight fast and 30 min after venous cannulation kept patent by slow infusion of isotonic saline.

Blood samples were taken in basal conditions (−15 and 0 min) and then every 15 min up to 120 min after drug administration. All samples from an individual subject were analyzed together for GH, PRL, ACTH and cortisol determination. Basal IGF-I levels were also assayed in all subjects.

Serum GH levels were measured in duplicate by IRMA (hGH-CTK IRMA, Sorin, Saluggia, Italy). The sensitivity of the assay was 0.15 µg/l. The inter- and intra-assay coefficients of variation were 2.9–4.5% and 2.4–4.0% respectively.

Serum PRL levels were measured in duplicate by IRMA (PRL-CTK IRMA, Sorin). The sensitivity of the assay was 0.5 µg/l. The inter- and intra-assay coefficients of variation were 3.9–6.8% and 3.3–7.5% respectively.

Plasma ACTH levels were measured by IRMA assay (Allegro HS-ACTH, Nichols Institute Diagnostic, San Juan Capistrano, CA, USA). The sensitivity of the assay was 1.0 pg/ml. The inter- and intra-assay coefficients of variation ranged from 6.9 to 8.9% and from 1.1 to 3.0% respectively.

Serum cortisol levels were measured by RIA (CORT-CTK 125, IRMA, Sorin). The sensitivity of the assay was 4.0 µg/l. The inter- and intra-assay coefficients of variation ranged from 6.6 to 7.5% and from 3.8 to 6.6% respectively.

Serum IGF-I levels were measured in duplicate by RIA (RIA, Nichols Institute Diagnostics). All samples were...
treated with acid–ethanol to avoid interference by binding proteins. The sensitivity of the assay was 0.1 μg/l. The inter- and intra-assay coefficients of variation were 10.1–15.7% and 7.6–15.5% respectively.

The hormonal responses are expressed either as absolute values or as areas under curves (AUC, 0–120 min) calculated by trapezoidal integration. Statistical analysis was carried out using a non-parametric ANOVA (Kruskal–Wallis) and then with a Mann–Whitney test, where appropriate.

Results are expressed as means ± S.E.M.

**Results**

Basal GH levels in Pre-C (2.0 ± 0.9 μg/l), Pub-C (1.5 ± 0.6 μg/l), Young (1.4 ± 0.8 μg/l) and Elderly (0.9 ± 0.3 μg/l) were similar, while IGF-I levels were different among the groups. In fact, IGF-I levels in Pre-C were lower than those in Pub-C (231.5 ± 47.4 μg/l, P<0.003), those in Young (208.5 ± 27.9 μg/l) were lower (P<0.001) than those in Pub-C, similar to those in Pre-C and higher (P<0.003) than those in Elderly (106.5 ± 9.0 μg/l). Basal PRL levels in Pre-C (5.6 ± 0.9 μg/l), Pub-C (4.5 ± 0.4 μg/l), Young (6.0 ± 0.7 μg/l) and Elderly (4.2 ± 0.4 μg/l) were similar. Basal ACTH and cortisol levels in Pre-C (17.1 ± 1.5 pg/ml and 100.6 ± 12.9 μg/l), Pub-C (25.5 ± 3.4 pg/ml and 102.9 ± 13.1 μg/l), Young (15.8 ± 1.6 pg/ml and 111.4 ± 7.1 μg/l) and Elderly (18.2 ± 2.9 pg/ml and 101.0 ± 8.0 μg/l) were similar.

The GH response to HEX was clear in Pre-C (peak, mean ± S.E.M., 22.0 ± 3.6 μg/l; AUC, mean ± S.E.M., 769.5 ± 122.2 μg*min/ml) but strikingly increased (P<0.001) in Pub-C (55.6 ± 7.1 μg/l; 1960.2 ± 283.5 μg*min/ml). The HEX-induced GH rise in Young (56.4 ± 7.9 μg/l; 1829.7 ± 243.1 μg*min/ml) persisted similar to that in Pub-C but then decreased in Elderly (30.5 ± 6.8 μg/l; 951.1 ± 232.9 μg*min/ml, P<0.005); the latter was, in turn, similar to that in Pre-C (Figs 1 and 3).

HEX induced a significant PRL increase which, however, showed no age-related variations, being similar in Pre-C (14.3 ± 3.0 μg/l; 512.1 ± 88.0 μg*min/ml), Pub-C (14.8 ± 2.8 μg/l; 584.0 ± 106.0 μg*min/ml), Young (13.1 ± 1.4 μg/l; 554.9 ± 56.0 μg*min/ml) and Elderly (12.3 ± 1.6 μg/l; 523.9 ± 59.6 μg*min/ml) (Figs 2 and 3).

The ACTH-releasing activity of HEX was present in Pre-C (33.9 pg/ml; 1356.6 ± 204.9 pg*min/ml) and was clearly enhanced (P<0.02) in Pub-C (57.3 ± 8.5 pg/ml; 2253.5 ± 242.8 pg*min/ml). The HEX-induced ACTH rise in Young (35.3 ± 4.9 pg/ml; 1258.1 ± 141.2 pg*min/ml) was lower (P<0.02) than that in Pub-C and similar to that in Pre-C. The ACTH response to HEX in Elderly (45.6 ± 9.4 pg/ml; 1786.5 ± 340.1 pg*min/ml) showed a further trend toward increase, being similar to that in Pub-C (Figs 2 and 3).

In contrast, the cortisol response to HEX showed no age-related variations, being similar in Pre-C (167.7 ± 23.7 μg/l; 7747.2 ± 1031.6 μg*min/ml), Pub-C (129.1 ± 17.6 μg/l; 6106.0 ± 862.9 μg*min/ml), Young (139.5 ± 10.5 μg/l; 6827.5 ± 509.6 μg*min/ml) and Elderly (155.8 ± 9.4 μg/l; 7950.6 ± 658.3 μg*min/ml). Among the various groups, no age-related differences in the timing of GH, PRL ACTH or cortisol peak occurrence were present (Figs 2 and 3).

**Side-effects**

HEX administration induced transient facial flushing in 40% of subjects and mild sleepiness in four Pre-C, five Pub-C, eight Young and three Elderly. No medication was required and no test had to be stopped.

**Discussion**

Our results demonstrate that in humans the GH- and ACTH-releasing activities of HEX undergo age-related variations which are dissociated, while the PRL-releasing activity of HEX is not dependent on age. Interestingly, the stimulatory effect of HEX on cortisol secretion is also not age-dependent and is dissociated by the age-related variations of the ACTH-releasing influence.

The strong GH-releasing activity but also a stimulatory effect on PRL, ACTH and cortisol secretion of GHRPs and non-peptidyl GHRP mimetics have been shown by many authors (1–9). We have previously demonstrated that the endocrine responses to HEX overlap with those to GHRP-2, another GHRP-6 super-analog (9); thus in discussing the effects of HEX we need to consider the effects of GHRPs.

Our present findings showing that the GH response to HEX varies across the lifespan agree with other data demonstrating that the GH response to GHRPs is
Figure 2
Mean (± S.E.M.) ACTH, cortisol and PRL responses to HEX (2.0 mg/kg i.v.) at various stages of the lifespan.
increased at puberty (3, 35), persists in adulthood and decreases thereafter, being reduced in aging by the sixth decade of life (3, 33, 36–39). This hormonal pattern is clearly different from that of GHRH. In fact, the most marked GHRH-induced GH secretion is present at birth while the somatotrope response to GHRPs in newborns is even lower than in prepubertal children (3). Moreover, while the GH response to GHRPs is clearly increased at puberty (35 and present data), the GHRH-induced GH rise in prepubertal and pubertal children is similar (35). This evidence indicates that the GH-releasing effect of GHRPs takes place via mechanisms, at least partially, different from those of GHRH, in agreement with other data in animals and in man (1, 3, 5, 40, 41). Interestingly, GHRPs release more GH than GHRH by puberty but not before (35). By adulthood, the somatotrope responsiveness to GHRPs, as well as that to GHRH, undergoes associated age-related variations, being clearly reduced in aging (3, 26, 33, 36–39 and present study). Variations during the lifespan in the neurohormonal and neurotransmitter control of somatotrope function (3, 26, 27) as well as in gonadal steroid levels (30) may influence the GH response to GHRPs, though the role of the putative endogenous GHRP-like ligand has to be taken into account (42). During puberty, the enhanced somatotrope responsiveness to GHRPs could depend on the increase in estradiol levels, as indicated by evidence that estradiol and testosterone, but not oxandrolone, augment the GH response to HEX in peripubertal children (43). On the other hand, the reduced GH responsiveness to GHRPs in aging does not depend on the fall of estrogens levels (36), but is probably due to the concomitant increased somatostatinergic and reduced GHRH activity. In fact, in elderly...

Figure 3 Age-related variations in the GH-, PRL- and ACTH-releasing activity of HEX.
subjects the maximal secretory capacity of somatotrope cells is fully restored only when HEX is coadministered with GHRH and arginine, which inhibits hypothalamic somatostatin release (3).

Data concerning the mechanisms underlying the PRL-releasing activity of GHRPs are scanty, though it has been hypothesized as an action at the pituitary level on somatotrophinoma cells. GHRP’s release PRL from human pituitary (24) and the GHRP-induced PRL rise is preserved in acromegalic patients bearing a somatomamotrope adenoma but not in patients bearing a pure prolactinoma (25). The evidence that HEX-induced PRL release does not vary with age suggests that the PRL-releasing effect of GHRPs is not influenced by estrogen levels and dopaminergic tone, which play a well-known role in the age-related variations of both spontaneous and stimulated PRL secretion (28).

More data are available about the mechanisms underlying the ACTH-releasing activity of GHRPs. The stimulatory effect of GHRPs on cortisol secretion seems to be due to their ACTH-releasing property, which takes place via a CNS-mediated action, as it is lost in the presence of hypothalamic-pituitary disconnection (20). Moreover, GHRPs do not stimulate ACTH release from rat pituitary (14). Some studies in animals and in humans led to the hypothesis that GHRPs act via a CRH-mediated action (7, 21) but our recent results favor the hypothesis that, in man, the stimulatory effect of GHRPs on the HPA axis is, at least partially, independent of both CRH and arginine vasopressin (AVP) (44). Our present findings showing that the ACTH-releasing activity of HEX increases at puberty, goes down in adulthood and then shows again a trend toward increase in aging, strengthen the hypothesis that a CRH-mediated mechanism does not mediate the ACTH-releasing activity of HEX. In fact, the CRH-induced ACTH response has been reported to be similar in prepubertal and pubertal children as well as in young adults (45). On the other hand, estrogens could play a role even in the increased corticotropic response to GHRPs at puberty; in fact, in animals there is evidence pointing to a role of sex steroids in the regulation of the HPA axis (31, 32). The increased corticotropic responsiveness to GHRPs in the elderly agrees with data showing that both spontaneous and CRH-stimulated ACTH secretion are increased in aging, probably as a consequence of altered sensitivity to glucocorticoid feed-back at the hippocampal level (23, 29).

In spite of clear age-related variations in the ACTH response, we did not find any age-related variations in the cortisol response to HEX. This is not surprising, considering that a striking rise in ACTH levels is not always followed by parallel cortisol increase after ACTH secretagogue administration (7, 23).

The age-dependent ACTH-releasing activity of GHRPs needs further investigation in order to understand better the neural control of the HPA axis in humans. Interestingly, the ACTH and cortisol responses to HEX are similar to those to hCRH and AVP and the HEX-induced ACTH and cortisol rises are exaggerated and strikingly higher than those after hCRH in patients with Cushings’s disease, in spite of their hypercortisolism (44, 46).

Above all, our present findings demonstrate that the age-related variations in the GH-, PRL- and ACTH-releasing activities of HEX are dissociated. This suggests that actions at different levels and/or on different receptor subtypes could underlie the endocrine effects of GHRPs, though, at present, there is no evidence for the existence of a specific GHRP receptor other than that already cloned (13). On the other hand, it has been demonstrated that specific GHRP binding sites are present in the human pituitary and hypothalamus, which play the main role in the control of the anterior pituitary, but also in other human CNS areas, including the hippocampus (1, 2), which plays an important role in the control of the HPA axis (23).

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References


