CLINICAL STUDY

Interaction between glucagon and Hexarelin, a peptidyl GH secretagogue, on somatotroph and corticotroph secretion in humans

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Abstract

Objective: Glucagon administration stimulates both somatotroph and corticotroph secretion in humans, although this happens only if glucagon is administered by the intramuscular route and not by the intravenous route. On the other hand, GH secretagogues (GHS) strongly stimulate GH and also possess ACTH-releasing activity.

Design and Methods: To clarify the mechanisms underlying the stimulatory effects of both glucagon and GHS on somatotroph and corticotroph secretion, we studied the GH, ACTH and cortisol responses to glucagon (GLU, 0.017 mg/kg i.m.) and Hexarelin, a peptidyl GHS (HEX, 2.0 µg/kg i.v.) given alone or in combination in 6 normal young volunteers (females, aged 26–32 years, body mass index 19.7–22.5 kg/m²).

Results: GLU administration elicited a clear increase in GH (peak vs baseline, mean $\pm$ S.E.M.: 11.6 ± 3.4 vs 3.3 ± 0.7 µg/l, $P < 0.02$), ACTH (11.6 ± 3.3 vs 4.1 ± 0.3 pmol/l, $P < 0.02$) and cortisol (613.5 ± 65.6 vs 436.9 ± 19.3 nmol/l, $P < 0.05$) levels. HEX induced a marked increase in GH levels (55.7 ± 19.8 vs 3.7 ± 1.9 µg/l, $P < 0.005$) and also significant ACTH (5.7 ± 1.1 vs 3.4 ± 0.6 pmol/l, $P < 0.01$) and cortisol (400.2 ± 31.4 vs 363.4 ± 32.2 nmol/l, $P < 0.05$) responses. The GH area under the curve (AUC) after HEX was clearly higher than after GLU (1637.3 ± 494.0 vs 479.1 ± 115.7 µg/l/120 min, $P < 0.04$) while HEX and GLU coadministration had a true synergistic effect on GH release (3243.8 ± 687.5 µg/l/120 min, $P < 0.02$). The ACTH and cortisol AUCs after HEX were lower ($P < 0.02$) than those after GLU (208.3 ± 41.3 vs 426.3 ± 80.9 pmol/l/120 min and $18\,874.5 \pm 1626.1$ vs $28\,338.5 \pm 2430.7$ nmol/l/120 min respectively). The combined administration of HEX and GLU had an effect which was less than additive on both ACTH (564.02 ± 76.5 pmol/l/120 min) and cortisol ($35\,424.6 \pm 5548.1$ nmol/l/120 min) secretion.

Conclusions: These results show that the intramuscular administration of glucagon releases less GH but more ACTH and cortisol than Hexarelin. The combined administration of glucagon and Hexarelin has a true synergistic effect on somatotroph secretion but a less than additive effect on corticotroph secretion; these findings suggest that these stimuli act via different mechanisms to stimulate somatotrophs while they could have a common action on the hypothalamo-pituitary-adrenal axis.

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Introduction

It is well known that glucagon administration induces clear increase in growth hormone (GH) and cortisol levels in humans (1–8). In fact, glucagon is considered a classical provocative stimulus of GH secretion for the diagnosis of GH deficiency (2–6) and it has been proposed as an alternative to insulin-induced hypoglycaemia for the diagnosis of adrenal insufficiency (5–7).

It has to be emphasized that glucagon stimulates somatotroph and corticotroph secretion after intramuscular but not after intravenous administration (9–13) indicating that glucagon *per se* is not a true GH and adrenocorticotrophin (ACTH) secretagogue. It has been hypothesized that intramuscular glucagon proteolysis could generate a peptidyl fragment endowed with GH- and ACTH-releasing activity (11, 13). The mechanisms underlying the stimulatory effect of intramuscular

Figure 1 GH response curves and AUCs to i.m. glucagon and Hexarelin administered alone or in combination to 6 normal subjects. * $P < 0.04$ vs glucagon alone, ** $P < 0.02$ vs glucagon or Hexarelin alone.

glucagon administration on both somatotroph and corticotroph secretion are unknown although there is evidence that it is unrelated to glucose variations and stress-mediated actions (1–3, 5, 10, 11, 13). Moreover, glucagon does not act at the pituitary level (11).

GH secretagogues (GHS) are peptidyl and non-peptidyl, non-natural molecules which possess potent stimulatory effects on somatotroph secretion, greater than that of GH releasing hormone (GHRH), both in animals and in humans (14–18).

GHS also possess an acute stimulatory effect on ACTH and cortisol secretion (14–20). Noteworthy, the ACTH- and cortisol-releasing activity of GHS is similar to that of human corticotrophin releasing hormone (hCRH) as well as to that of arginine vasopressin (AVP) and naloxone, an opioid antagonist (19, 21–25).

GHS act via specific receptors and this evidence suggested the existence of a natural GHS-like ligand (26–29). Indeed, an endogenous ligand specific for GHS receptors (GHS-R) endowed with a stimulatory effect on GH secretion, named Ghrelin, has recently been identified in both rat and human stomach (30). More recently, a second GHS-R natural ligand has been purified from rat stomach; it derives from alternative splicing of the Ghrelin gene and has been named des-Gln14-Ghrelin (31). The GH-releasing activity of GHS is mediated by actions at the pituitary and, mainly, at the hypothalamic level, probably via antagonism of the somatostatinergic activity and enhanced activity of GHRH secreting neurons (32–37). On the other hand, at least in physiological conditions, the ACTH-releasing activity of GHS depends on a CNS-mediated action. Data in animals and in humans suggested that the stimulatory effect of GH releasing peptides (GHRPs) on ACTH secretion could be mediated by CRH and/or AVP although an action independent of both these neuro-hormones has also been hypothesized (21, 38–40).

Based on the foregoing, in the present study we compared the effects of glucagon and Hexarelin, a peptidyl

GH secretagogue, and studied their interaction on GH, ACTH and cortisol levels in normal young volunteers.

Subjects and methods

Peptides and drugs

Vials containing 1 mg glucagon were purchased from Novo Nordisk (Rome, Italy). Vials containing 100 µg lyophilized Hexarelin (HEX) were kindly provided by Europeptides (Argenteuil, France).

Study design

Six normal young women (aged 26–32 years, body mass index 19.7–22.5 kg/m²) in the early follicular phase of the cycle were studied. The study has been approved by the independent Ethical Committee of the University of Turin and informed consent was obtained from all subjects.

All subjects underwent the following treatments, at least three days apart: (1) i.m. glucagon (GLU, 0.017 mg/kg i.m. as a bolus at 0 min) followed by i.v. placebo (1.0 mg saline at +90 min); (2) i.m. placebo (at 0 min) followed by i.v. Hexarelin (HEX, 2.0 µg/kg i.v. as a bolus at +90 min); (3) i.m. GLU (0.017 mg/kg i.m. as a bolus at 0 min) followed by i.v. HEX (2.0 µg/kg i.v. as a bolus at +90 min).

The tests started between 0730 and 0800 h after an overnight fast, and 30 min after venous cannulation kept patent by slow infusion of isotonic saline.

Blood samples for GH, ACTH, cortisol and glucose measurements were taken at 30-min intervals from 0 to +90 min and at 15-min intervals from +90 to +210 min. All samples from an individual subject were analysed at the same time.

Serum GH levels were measured in duplicate by an immunoradiometric assay (hGH-CTK IRMA, SORIN, Saluggia, Italy). The sensitivity of the assay was 0.15 µg/l. The inter- and intra-assay coefficients of

Figure 2 ACTH and cortisol response curves and AUCs after i.m. glucagon and Hexarelin administered alone or in combination to 6 normal subjects. * $P < 0.02$ vs glucagon alone and glucagon+Hexarelin.

variation ranged from 2.9 to 4.5% and from 2.4 to 4.0% respectively. Plasma ACTH levels were measured in duplicate by an immunoradiometric assay (Allegro HS-ACTH, Nichols Institute Diagnostic, San Juan Capistrano, CA, USA). The sensitivity of the assay was 0.02 pmol/l. 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 in duplicate by a radioimmunoassay (CORT-CTK 125, IRMA, SORIN, Saluggia, Italy). The sensitivity of the assay was 11.4 nmol/l. The inter- and intra-assay coefficients of variation ranged from 6.6 to 7.5% and from 3.8 to 6.6% respectively. Plasma glucose levels (nmol/l) were measured by a gluco-oxidase colorimetric method (Menarini Diagnostic, Florence, Italy).

The hormonal responses (mean $\pm$ S.E.M.) are expressed as absolute levels and as areas under the response curve (AUC, from +90 to +210 min).

The statistical analyses were carried out using a non-parametric ANOVA (Freedman test) followed by Wilcoxon test.

Results

Intramuscular GLU administration elicited a clear increase in GH (peak vs baseline, mean $\pm$ S.E.M.: 11.6 ± 3.4 vs 3.3 ± 0.7 $\mu\text{g/l}$, $P < 0.02$), ACTH (11.6 ± 3.3 vs 4.1 ± 0.3 pmol/l, $P < 0.02$) and cortisol (613.5 ± 65.6 vs 436.9 ± 19.3 nmol/l, $P < 0.05$) levels.

HEX induced a marked rise in GH levels (55.7 ± 19.8 vs 3.7 ± 1.9 $\mu\text{g/l}$, $P < 0.005$) and also significant increases in ACTH (5.7 ± 1.1 vs 3.4 ± 0.6 pmol/l, $P < 0.01$) and cortisol (400.2 ± 31.4 vs 363.4 ± 32.2 nmol/l, $P < 0.05$) levels (Figs 1 and 2).

The GH AUC after HEX administration was clearly higher than after GLU administration (1637.3 ± 494.0

vs 479.1 ± 115.7 $\mu\text{g/l/120 min}$, $P < 0.04$). Coadministration of HEX and GLU had a true synergistic effect on GH release (3243.8 ± 687.5 $\mu\text{g/l/120 min}$, $P < 0.02$ vs the arithmetic sum of the hormonal responses to HEX and GLU alone) (Fig. 1).

The ACTH and cortisol AUCs after HEX administration were lower ($P < 0.02$) than those after GLU administration (208.3 ± 41.3 vs 426.3 ± 80.9 pmol/l/120 min and $18\,874.5 \pm 1626.1$ vs $28\,338.5 \pm 2430.7$ nmol/l/120 min). The ACTH and cortisol responses to the combined administration of HEX and GLU (564.02 ± 76.5 pmol/l/120 min and $35\,424.6 \pm 5548.1$ nmol/l/120 min) were significantly higher ($P < 0.05$) than after HEX alone but were not different from those following GLU (Fig. 2).

GLU administration induced a biphasic variation in blood glucose levels: a significant increase was recorded at +30 min (5.9 ± 0.2 vs 4.1 ± 0.14 nmol/l, $P < 0.05$) followed by a decrease at 180 min (3.19 ± 0.2 nmol/l, $P < 0.05$). The glucagon-induced blood glucose variations were not modified by HEX.

Side effects

The administration of GLU i.m. induced slight, transient nausea in all subjects. HEX administration induced transient facial flushing in all subjects and mild somnolence in two of them. The combined administration of the two substances did not modify the effects recorded after the administration of each substance alone.

Discussion

The results of the present study show that the intramuscular administration of glucagon releases less GH but more ACTH and cortisol than Hexarelin, a

peptidyl GH secretagogue. The combined administration of glucagon and Hexarelin has a true synergistic effect on somatotroph secretion and an effect less than additive on corticotroph secretion.

The stimulatory effect of the intramuscular glucagon administration on GH secretion and its usefulness for the diagnosis of GH deficiency are well known (1–8). Our present results confirm the strong GH-releasing effect of i.m. glucagon, even if this was clearly lower than that of Hexarelin which, like all other GHS, is one of the most potent stimuli of somatotroph secretion (17, 41, 42).

The mechanisms underlying the stimulatory effect of i.m. glucagon administration are, however, largely unclear. Evidence that the intravenous administration of the hormone does not affect GH secretion indicates that glucagon *per se* does not possess GH-releasing activity (9–13). It has been hypothesized that intramuscular glucagon proteolysis could generate a peptidyl fragment endowed with true GH-releasing activity (11, 13). The mechanisms underlying this stimulatory effect are unrelated to glucose variations and stress-mediated actions (1, 5, 10, 11, 13). Moreover, they should not involve GHRH- and somatostatin (SS)-mediated actions. In fact, the stimulatory effect of i.m. glucagon is synergistic with that of GHRH (11) but is also potentiated by beta-adrenergic blockers, which act via inhibition of hypothalamic SS release (43, 44).

The strong GH-releasing activity of GHS involves actions at the pituitary and, mainly, at the hypothalamic level (14). GHS, at least partially, act as functional SS antagonists (32, 33, 45, 46). The stimulatory effect of GHS on GH secretion is partially refractory to inhibitory influences including neurotransmitters, glucocorticoids, glucose, lipids and even exogenous recombinant human GH and somatostatin (17, 46). Moreover, even at very low dose GHS truly synergize with GHRH (47, 48) indicating different mechanisms of action for these peptides. However, GHS need the integrity of the hypothalamo-pituitary unit and, particularly, of GHRH activity. In fact, the GH-releasing effect of GHS is strongly reduced in the presence of hypothalamo-pituitary disconnection as well as by a GHRH antagonist and is totally lacking in patients with GHRH receptor deficiency (34, 36, 37, 41, 49). Whether the mechanisms of action underlying the effect of synthetic GHS really reflect the activity of Ghrelin, the probable natural ligand of GHS-R, has still to be clarified (30, 31).

Apart from the interaction with GHRH and insulin-induced hypoglycaemia (17, 50), the GH-releasing effect of GHS in humans is generally unaffected by several substances known to potentiate the GHRH-induced GH secretion such as arginine, pyridostigmine, clonidine and atenolol (45, 51). This evidence makes the true synergism between i.m. glucagon and Hexarelin on the GH response the more impressive; in fact, the response was similar to the synergistic effect of GHS and GHRH (45, 47, 50).

The synergism between i.m. glucagon and Hexarelin on GH secretion indicates that the substances act via different mechanisms. These are unlikely to include glucose variations; in fact, the synergistic effect took place well before the late decrease in blood glucose induced by glucagon.

As both Hexarelin and i.m. glucagon synergize with GHRH (11, 45, 47, 50), their interaction is unlikely to be dependent on a GHRH-mediated mechanism. The functional SS antagonism of GHS could lead to enhancement of the somatotroph response to i.m. glucagon; in fact, the stimulatory effect of glucagon is also enhanced by propranolol which acts via inhibition of hypothalamic somatostatin release (43, 44).

Our findings also show that i.m. glucagon stimulates the hypothalamo-pituitary-adrenal (HPA) axis to a greater extent than Hexarelin, which is able to induce corticotroph responsiveness similar to that elicited by AVP, CRH and naloxone (21, 23, 24, 39). This strengthens the potency of i.m. glucagon as a provocative test alternative to insulin-induced hypoglycaemia for the diagnosis of adrenal insufficiency (5–7).

The ACTH-releasing activity of GHS mainly depends on a CNS-mediated action, at least under physiological conditions; in fact, GHS do not stimulate ACTH secretion from normal animal and human pituitary cell cultures (52–54) nor after pituitary stalk lesion (55, 56).

CRH- and AVP-mediated actions of GHS have been suggested to occur in animals (39, 40). In humans, it has been shown that Hexarelin has no interaction with AVP and has an effect less than additive with hCRH (21, 39). These findings indicate that, in man, CRH is unlikely to mediate the ACTH-releasing effect of GHRPs which, in turn, could act directly or indirectly via AVP or, alternatively, via mechanisms independent of both CRH and vasopressin. In fact, neuropeptide Y and gamma aminobutyric acid could play a major role in the ACTH-releasing activity of GHS (35, 57).

The less-than-additive effect of Hexarelin and i.m. glucagon on corticotroph secretion suggests the possibility that they act via a common mechanism(s). Interestingly, evidence that, at least in rats, Ghrelin, a natural GHS-R ligand, shows activity fully specific for GH without any stimulatory effect on ACTH and cortisol release (30) could indicate that Ghrelin is not involved in the stimulatory effect of glucagon on the activity of the HPA axis.

In conclusion, this study shows that the intramuscular administration of glucagon releases less GH but more ACTH and cortisol than Hexarelin, a peptidyl GH secretagogue. Their synergistic effect on somatotroph secretion suggests different mechanisms of action.

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