CLINICAL STUDY

Ghrelin-induced GH secretion in normal subjects is partially resistant to homologous desensitization by GH-releasing peptide-6

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Abstract

Objective: GH secretagogues and GH-releasing hormone (GHRH) exert a complex cross-talk at the somatotrope cell, and undergo homologous and heterologous desensitization. On the other hand, the discovery of ghrelin as a new factor implicated in the regulation of GH secretion makes a thorough assessment of its properties and cell biology processes mandatory. In order to implement this, three different testing schedules were devised using the administration, on the same day, of two GH stimuli administered in sequential order 120 min apart. The two aims of the study were (a) to evaluate the relative potency of ghrelin in comparison with other GH stimulants and (b) to assess the presence of homologous or heterologous desensitization between these compounds.

Design: The different testing days performed in random order were (a) on one day, saline was administered at time 0 min and ghrelin at time 120 min, (b) on another testing day, GHRH was administered at 0 min followed by ghrelin at 120 min and (c) on the last testing day, GH-releasing peptide-6 (GHRP-6) and ghrelin were injected at 0 and 120 min respectively. Ghrelin, GHRH and GHRP-6 were always administered at 1 μg/kg i.v., and plasma GH was measured.

Patients: Six normal subjects participated in the study after providing informed consent, and each was assessed on three different testing days, at least 1 week apart.

Results: Saline did not modify peak GH (means±S.E.) values (1.5±0.6 μg/l), and ghrelin administered 120 min later induced a significant GH rise (39.9±2.8 μg/l). On a different testing day, GHRH induced a GH peak (9.4±2.8 μg/l) lower than that of ghrelin injected 120 min later (26.8±4.7 μg/l). On the last testing day, GHRP-6 at time 0 induced a GH peak of 18.4±5.9 μg/l and ghrelin 120 min later a peak of 19.8±2.9 μg/l. The ghrelin-mediated GH secretion after GHRP-6 was significantly lower than the GH elicited by ghrelin when the preceding administration was saline. This demonstrated that ghrelin was partially affected by GHRP-6 and was not affected by GHRH.

Conclusions: Calculated at equal mass doses or in molecular terms, ghrelin appears to be a more potent stimulus than GHRP-6 and GHRH. Ghrelin was completely insensitive to the previous administration of GHRH as well as relatively resistant to the homologous desensitization exerted by GHRP-6.

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Introduction

Growth hormone (GH) is tightly regulated by the metabolic milieu, a regulation that is superimposed over the classical peripheral regulation by thyroid, gonadal, glucocorticoid and peptide hormones (1). GH is the only known pituitary hormone regulated by at least two antagonistic hypothalamic hormones, GH-releasing hormone (GHRH) and somatostatin, plus a liver-derived hormone, insulin-like growth factor-I, which exerts a classical negative feedback on GH. This picture points towards the fact that GH is one hormone whose actions are implicated in a dual action on somatic growth and in the regulation of general metabolism, and which is, in turn, regulated by the energetic homeostasis of the individual. This dual field of action and regulation currently seems to be explained by a newly discovered hormone, ghrelin, which may well be the bridge connecting somatic growth and body composition with the general metabolism (2, 3).
Ghrelin is the end-point of a search that started with the developing of artificial compounds such as the highly potent GH-releasing hexapeptide, called GHRP-6 (4, 5), with no parallel structure in nature. GHRP-6 is a potent GH releaser both in vitro and in vivo (6), in all species tested so far, and was the basis for the subsequent synthesis of other types of compounds with similar properties, such as hexarelin (7) or MK-0677 (8), all subsequently known as GH secretagogues (GHS). The GHSs were used for the cloning of the GHS receptor (9), and the last milestone in the field has been the isolation of the endogenous or natural ligand of the GHS receptor, a peptide called ghrelin, which releases GH both in vivo and in vitro in a dose-dependent manner (10). Surprisingly, ghrelin was isolated from the stomach, where its expression is higher than in any other tissue. Ghrelin represents the natural hormone of a new physiological system implicated in GH regulation (10), and also in the regulation of energy homeostasis (11).

Ghrelin releases GH in vivo (12) and also when administered directly via an intracerebroventricular route (13) and, furthermore, it is able to enter the central nervous system from the periphery (14). Then, although yet to be proven, it may well be the case that stomach-derived ghrelin participates physiologically in GH regulation. Although it is possible to antic- plate that ghrelin will share many of the properties previously communicated for GHSs, it is obvious through experimental and theoretical data that ghrelin activates one of the GHS receptors but probably not the only one. In fact, in some biological systems there are marked differences between the effects of ghrelin and those elicited by other GHSs. These discrepancies are thought to be due to the existence of more than one GHS receptor with different affinities for ghrelin and GHSs. Careful analysis of the mechanisms of the action of ghrelin is therefore currently mandatory (15).

It has previously been reported that the administration to normal volunteers of GHRH followed 2 h later by a GHS maintains the GH-releasing capability of both stimuli without mutual interference (16). On the contrary, changing the order of the stimuli, the previous administration of a GHS similar to GHRP-6 completely blunts the subsequent action of GHRH or of a second administration of GHRP-6 (16, 17). This peculiar cross-talk between stimuli reflects the fact that GHSs exert homologous desensitization on the GHS receptor, as well as heterologous desensitization on the GHRH receptor at the somatotrope cell. The cell biology basis of that behaviour has not yet been explained.

In the present work, an experimental design previously shown to be effective for testing inter-receptor desensitization in man was used to further understand the intimate mechanism of the action of ghrelin. The two aims of the work were (a) to assess the potency of ghrelin in normal volunteers compared with other GH stimuli and (b) to assess if GHRP-6 exerts homologous desensitization on the GH secretion elicited by ghrelin.

Subjects and methods
Six normal male volunteers, aged 32±3 years (range 22–42 years) participated in this study after providing informed consent. All had normal life styles, were taking no medication and were within 10% of their ideal body weight. The study was approved by the Hospital Bioethics Committee, Belgrade University.

Each subject underwent three experimental double tests on three different days, in random order at least one week apart. On each day, subjects were challenged twice with different GH stimulants, in this manner each subject served as his own control. Tests were started at 0800 h after an overnight fast, with the subjects recumbent. An indwelling catheter was placed in a forearm vein and kept patent with a slow infusion of 150 mmol/l NaCl. The first blood sample was obtained at −30 min and additional blood samples were obtained at appropriate intervals over the following 4.5 h of testing. After centrifugation, plasma was kept frozen until assay.

On one experimental day, subjects were given saline at 0 min and at 120 min they were given ghrelin (Eur-opeptides, Argenteuil, France) at a dose of 1 μg/kg i.v.; this design was called the saline–ghrelin test. On the second experimental day, subjects were given GHRH (GHRH 1–29 NH2; Geref, Serono, Madrid, Spain) at a dose of 1 μg/kg i.v. at 0 min and at 120 min they were given ghrelin at a dose of 1 μg/kg i.v. (GHRH–ghrelin test). On the third experimental day, subjects were given GHRP-6 (Cilag/ Laufelfinger, Switzerland) at a dose of 1 μg/kg i.v. at 0 min followed at 120 min by ghrelin at a dose of 1 μg/kg i.v. (GHRP-6–ghrelin test).

Serum GH concentrations were determined using a time-resolved fluorooimmunoassay (Delfia; Wallac Oy, Turku, Finland) with a GH sensitivity of 0.011 μg/l and coefficients of variation of 6.3% (0.4 μg/l), 5.3% (10.2 μg/l) and 4.2% (43.4 μg/l). All samples from each group were analyzed in the same assay run. Hormone levels are presented and analyzed as absolute values or as the mean GH peak (means±S.E.). The areas under the curve (AUC) were calculated by trapezoidal integration. Data were analyzed by ANOVA of repeated measured and afterwards a comparison between groups was performed by ANOVA of repeated measures. The statistical level of significance was set at P < 0.05.

Results
In the saline–ghrelin test, saline administration did not modify GH basal levels, as expected; the mean GH peak being 1.5±0.6 μg/l. The administration of ghrelin at
120 min elicited a significant GH mean peak of $39.9 \pm 2.8 \mu g/l$ (Fig. 1). In the GHRH–ghrelin test, the GH secretion elicited by GHRH was rather erratic with a mean GH peak of $9.4 \pm 2.8 \mu g/l$, and when ghrelin was administered 120 min later the GH mean peak was $26.8 \pm 4.7 \mu g/l$. In the GHRP-6–ghrelin test, GHRP-6 induced a mean GH peak of $18.4 \pm 5.9 \mu g/l$, while ghrelin at 120 min induced a GH mean peak of $19.8 \pm 2.9 \mu g/l$. When the AUC values were calculated and expressed as µg/l per 60 min, the values were $39\pm 24$ and $1669\pm 135$ for saline–ghrelin, for GHRH–ghrelin they were $405\pm 122$ and $1028\pm 169$ and finally they were $698\pm 246$ and $760\pm 118$ for GHRP-6–ghrelin.

A comparison between the mean GH peaks elicited (Fig. 2) showed that all stimuli elicited a significantly greater GH secretion than either saline or GHRH. Interestingly, ghrelin was a more effective GH releaser ($P = 0.0002$) than GHRH, and also more potent ($P = 0.019$) than GHRP-6 when both were administered at equal mass dose. On the other hand, while the previous administration of GHRH did not alter ghrelin’s capability for releasing GH, GHRP-6 did induce a significant desensitization, being the difference between the two ghrelin administrations (preceded by saline or preceded by GHRP-6) of $P = 0.004$. Similar results were obtained when AUC values were compared instead of peaks (data not shown).

No side-effects were reported in any stimulation test.

**Discussion**

Ghrelin is a stomach-derived, 28 amino acid peptide that might well be a new hormone implicated in the regulation of GH secretion. In the present work, it has been shown that ghrelin at equal mass doses is more effective or potent than either GHRH or GHRP-6, a fact of practical and theoretical relevance (Table 1). As we used the biologically active core of GHRH, i.e. GHRH 1–29, a relevant practical finding was that ghrelin and GHRH 1–29 have similar molecular weights, while that of GHRP-6 is considerably (25%) lower. At a similar µg/kg dose, there was a similar administration of ghrelin and GHRH molecules, showing the extraordinary difference in the potency of these compounds. The fact that ghrelin was twice as potent as GHRP-6 is remarkable, even considering that four GHRP-6 molecules for each molecule of ghrelin was given. It has previously been shown, and appears to be confirmed by the data reported herein, that GHRP-6 was more efficacious but less potent than GHRH, even when considering the erratic pattern of GH secretion typical of GHRH administration (6, 18). In fact (Table 1), on a µg/kg injection we administered four times more GHRP-6 molecules than GHRH 1–29 molecules while the GH released by GHRP-6 was only twice that of GHRH. Hence, from the first analysis it may be concluded that the order of potency to release GH is ghrelin > GHRH > GHRP-6, data which are at
odds with common thinking but in accordance with previous reports (19–23). The second relevant observation was that a previous injection of saturating doses of GHRP-6 partially, but not totally, desensitized the ghrelin-mediated GH secretion. This means that ghrelin, in normal individuals, is partially resistant to a previous administration of GHRP-6 and to the ensuing GH rise.

The cross-talk between different receptors of a given cell is a well-established fact in the field of cell biology, and implies that the previous activation of a receptor is able to modulate the response of a subsequently activated one, by enhancing, reducing or modifying the response pattern. In the ghrelin–GHS field of research, two types of cellular responses have been studied up to now: the homologous desensitization effects and the cross-talk that is observed between the GHRH receptor and the GHS receptor. In vivo, GHRH activation of its receptor on somatotrope cells is necessary to allow GHSs to be fully operative, and in experimental neutralization of GHRH or in natural conditions with impeded GHRH activity, GHSs are no longer releasing GH (24, 25). Thus, GHRH exerts a permissive action over GHS activity, but saturating doses of GHRH have no negative effects on a later administration of GHS (16). In contrast, a previous administration of GHSs such as hexarelin or GHRP-6 leads, 2 h later, to a near complete blockade of GHRH-mediated GH secretion, a finding called heterologous desensitization (16, 17).

Homologous desensitization has been described in vivo for GHRH-mediated GH secretion, and, as expected, a first administration of GHRH leads to a reduced or absent GH response to a second GHRH dose, providing that the two challenges are separated in time by only a few hours (16). Similar results are observed when hexarelin, GHRP-6 or any other GHS is sequentially administered (17). The first GHS dose induces a large GH secretion, while the second GHS dose administered 2 h later has severely reduced GH secretion. The basis of the homologous desensitization phenomenon is most probably a down-regulation of the cell membrane receptor, which is internalized after activation, and requires more than 2 h before being recycled towards the membrane again. In the case of the homologous desensitization to sequential administration of GHRH, the GH rise elicited in the first stimulation may have a negative action over the second one, most probably through a hypothalamic somatostatinergic discharge (26). However, this explanation is unlikely for GHSs that are relatively resistant to the inhibitory action of a previous GH rise (16, 27). In the present work, it was observed that a previous GH rise elicited by GHRH was unable to modify the ghrelin stimulatory activity observed 2 h later, thus ghrelin stimulation shares with the artificial GHS the property of being relatively insensitive to GHRH as well as to a previous GH rise. However, and different from the facts reported for artificial GHS in which a first saturating dose erases the GH release of a second dose, in the present work it has been observed that ghrelin was partially insensitive to the GHRP-6 administered previously. This finding adds support to the view that GHSs such as hexarelin and GHRP-6 activate receptors that are similar, but not necessarily identical, to those activated by ghrelin (28–31). When the AUC values in this study were considered (1669 for saline–ghrelin, 405 and 760 for GHRP-6–ghrelin) it appears that the sums of all these stimuli seem to be similar. Although a possible depletion of GH stores was not ruled out in the present work, such an interpretation is unlikely because the GH releasable

Table 1 The molecular weights (MW) and potency of ghrelin, GHRH 1–29 and GHRP-6 at similar dose levels.

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<tr>
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<th>Potency (μg/kg)</th>
<th>Absolute potency/mol (%)</th>
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<tbody>
<tr>
<td></td>
<td>MW (%)</td>
<td>GH peak</td>
</tr>
<tr>
<td>Ghrelin</td>
<td>3315 (100)</td>
<td>39.9</td>
</tr>
<tr>
<td>GHRH 1–29</td>
<td>3358 (100)</td>
<td>9.4</td>
</tr>
<tr>
<td>GHRP-6</td>
<td>873 (25)</td>
<td>18.4</td>
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Ghrelin-induced GH secretion and GHRP-6

Ghrelin-induced GH secretion and GHRP-6

References

Pool largely exceeds (32, 33) the values here reported. Although it is tempting to assume that properties previously observed for artificial GHSs may be automatically transferred to ghrelin, it cannot be taken for granted. Ghrelin demonstrates new and surprising properties and a distribution that makes the compound unique (34–38). The whole physiology and pathophysiology of that new system regulating GH secretion and energy homeostasis needs to be assessed and described carefully. The similarities and differences between ghrelin and artificial GHSs and the peculiarities of their respective cross-talk may help (a) to clarify the doubts regarding the existence of receptor subtypes and (b) to better understand the basis of the somatotrope cell biology and the mechanisms of its derangement.

In conclusion, with the model of sequential administration separated by 2 h it has been observed that ghrelin is the more potent releaser of GH and, on a molar basis, the order of potency should be ghrelin > GHRH > GHRP-6. On clinical grounds, GHRP-6 is less potent but more efficacious at releasing GH. The ghrelin-stimulating capability over GH is partially insensitive to a previous GH rise but is also insensitive to the previous administration of either GHRH or GHRP-6.

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