From Growth Hormone-Releasing Peptides to Ghrelin: Discovery of New Modulators of GH Secretion

revisão

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ABSTRACT

Growth hormone (GH)-releasing hormone and somatostatin modulate GH secretion. A third mechanism has been discovered in the last decade, involving the action of GH secretagogues. Ghrelin is a new acylated peptide produced mainly by the stomach, but also synthesized in the hypothalamus. This compound increases both GH release and food intake. The relative roles of hypothalamic and circulating ghrelin on GH secretion are still unknown. Endogenous ghrelin might amplify the basic pattern of GH secretion, optimizing somatotroph responsiveness to GH-releasing hormone. This peptide activates multiple interdependent intracellular pathways at the somatotroph, involving protein kinase C, protein kinase A and extracellular calcium systems. However, as ghrelin induces a greater release of GH *in vivo*, its main site of action is the hypothalamus. In this paper we review the available data on the discovery of ghrelin, the mechanisms of action and possible physiological roles of GH secretagogues and ghrelin on GH secretion, and, finally, the regulation of GH release in man after intravenous administration of these peptides. **(Arq Bras Endocrinol Metab 2006;50/1:17-24)**

Keywords: Ghrelin; GH; Growth hormone secretagogues; GHS

RESUMO

Dos Peptídeos Liberadores do Hormônio de Crescimento (HC) à Ghrelina: Descoberta de Novos Moduladores da Secreção de HC

A secreção de hormônio de crescimento (HC) é modulada pelo hormônio liberador de HC e pela somatostatina. Na ultima década foi descoberto um terceiro mecanismo de controle, envolvendo os secretagogos de HC. A ghrelina é um peptídeo acilado, descoberto recentemente, que é produzido no estômago, porém também é sintetizado no hipotálamo. Este peptídeo é capaz de liberar HC, além de aumentar a ingestão alimentar. A ghrelina endógena parece amplificar o padrão básico de secreção de HC, ampliando a resposta do somatotrófo ao hormônio liberador de HC. Este peptídeo estimula múltiplas vias intracelulares interdependentes no somatotrófo, envolvendo a proteína quinase C, proteína quinase A e sistemas moduladores de cálcio extracelular. Entretanto, como a liberação de HC induzida pela ghrelina *in vivo* é mais acentuada que *in vitro*, seu local de atuação predominante é no hipotálamo. Nesse artigo apresentamos uma revisão sobre a descoberta da ghrelina, os dados existentes sobre os mecanismos de ação e possível papel fisiológico dos secretagogos de HC e da ghrelina na secreção de HC e, finalmente, os efeitos da administração endovenosa destes peptídeos sobre a secreção de HC no homem. **(Arq Bras Endocrinol Metab 2006;50/1:17-24)**

Descritores: Ghrelina; GH; Secretagogos de hormônio de crescimento; GHS

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Historical review

BEFORE THE IDENTIFICATION of the growth hormone-releasing hormone (GHRH), Bowers et al. (1) discovered a group of synthetic compounds with growth hormone (GH)-releasing properties (for review see ref. 2). These substances were developed from the met-enkephalin molecule, through theoretical calculations, computer modeling, chemical alterations and studies of biological activity. These small peptides were able to induce a weak GH release initially. Further chemical changes led to the synthesis of more potent compounds, including peptides such as GH releasing peptide-6 (GHRP-6), GHRP-2, hexarelin, and non-peptides, as MK-0677, which could be administered orally (2). In the last decade several studies were performed with these growth hormone secretagogues (GHS), especially with GHRP-6, and the obtained data have suggested that these compounds are modulators of GH release (3). It was shown that GHS enhance GH secretion by different mechanisms than those activated by GHRH (for reviews see ref. 2,3), and these substances act through different receptors than those of GHRH, somatostatin or opioid peptides. In 1996 Howard et al. cloned the GHS receptor (GHS-R), which was mainly found in the anterior pituitary and in the hypothalamus, and also in other areas of the central nervous system (4). In 1999 Kojima et al. discovered the endogenous ligand for these orphan receptors in the stomach, and this new hormone was denominated ghrelin (from *ghre*, the Indo-European root of the word grow) (5). Ghrelin is also present in small amounts in the hypothalamus and is able to stimulate GH release in a potent manner (5,6). This peptide is a new member of the brain-gut peptide family, and it acts in the control of appetite, an effect that is independent of GH release (for review see ref. 7,8). Ghrelin might have other actions, which are currently being investigated (7,8). The discovery of ghrelin is an example of reverse pharmacology: the chemical synthesis of compounds, such as GHS, led to the discovery of the endogenous orphan receptor and, finally, to the isolation of its natural ligand.

GHS receptor

Howard et al. cloned the GHS receptor in 1996 and found that it belongs to the G-protein family. The GHS receptor has seven transmembrane spanning segments and three intracellular and extracellular loops (4). Two subtypes of receptors were discovered, GHS-R1a, which is active, and GHS-R1b, a shorter isoform, which apparently does not have biological activity (4). It is likely that other subtypes might exist. The human GHS-R1a has 366 aminoacids and is highly conserved in evolution. The active receptor was found in the anterior pituitary and in the hypothalamus, as well as in other regions of the central nervous system (4). GHS-R1a is present in several hypothalamic areas, including the arcuate, ventromedial and paraventricular nuclei (2,4). GHS-R might modulate biological rhythms, memory, mood, learning and appetite (2). In the pituitary GHS-R was found exclusively in somatotrophs (2). In knockout mice for GHS-R1a ghrelin fails to increase both GH release and food intake, indicating that both actions of ghrelin are dependent on this type of receptor. (9). GHS-R1a was also found in other tissues such as pancreas, heart, adrenal gland and the thyroid (10). It is interesting that GHS-R1b, the inactive form, has a widespread distribution in peripheral tissues but its function has not been elucidated (10).

Ghrelin

In 1999 Kojima et al. surprisingly found a major increase in intracellular calcium concentrations with the addition of stomach extracts to an *in vitro* system of cells, which expressed GHS-R1a (5). Further studies led to the isolation of a 28-aminoacid peptide with a fatty acid chain modification (n-octanoic acid), in the serine 3 residue. This hydrophobic compound, which is the first known natural bioactive peptide modified by an acyl acid, was called ghrelin. It was also found that ghrelin and the GHS, such as GHRP-6, have no structural similarity, which is quite intriguing (5). The post-translational fatty acid chain modification (n-octanoyl residue) is essential for the biological activity of ghrelin, including GH release and appetite stimulation. Shorter fragments, with the first four to five residues, are also able to stimulate signal transduction of GHS-R1a *in vitro* when they have an intact acylated serine (7). However, the main circulating form is nonacylated ghrelin, which might have non-endocrine actions (5). Circulating nonacylated ghrelin levels are reduced by 80% after gastrectomy or gastric bypass in humans, demonstrating that this peptide is mainly produced in the stomach (11). It was recently shown that ghrelin crosses the blood brain barrier, and this transport occurs in both directions, from the central nervous system to blood and from blood to brain (12). It has also been shown that the acyl residue is important for this transport (12). The gene that encodes ghrelin is located on chromosome 3 in men and encodes a precursor of 117 aminoacids, with an 82% homology within species (5). Two isoforms of mRNA of prepro-ghrelin are produced by the same gene, by alternative splicing, in the stomach (5). One encodes

the ghrelin precursor while the other encodes des-Gln^{14} ghrelin precursor, which has no glutamine on position 14 (5). This latter peptide has 27 aminoacids and is biologically active, but is present in small amounts. Therefore, the main active form is ghrelin. Ghrelin is found in the submucosal layer of the stomach fundus, in endocrine oxyntic cells (X/A), and also, in lower concentrations, in the gastrointestinal tract (7). Both ghrelin and its mRNA are present in the arcuate nucleus of the hypothalamus and in the pituitary gland (5,10). At pituitary level it might act in autocrine or paracrine manner. It has been recently shown that ghrelin is expressed in lactotrophs, somatotrophs and thyrotrophs, cells that are dependent, for differentiation, on Pit 1 gene expression (8). Ghrelin is also able to modulate Pit-1 transcription. Ghrelin has a widespread distribution and has been found in the lung, kidney, ovary, testis, placenta, among others, but its physiological role in these tissues remains to be elucidated (10). Because the localization of the biological active receptor (GHS-R1a) is not the same as the peptide, it is likely that other receptor subtypes might exist (10). Ghrelin is found in considerable amounts in circulation and this peptide has several actions apart from its modulatory role on GH release (7,8). Ghrelin enhances food intake, by activation of NPY/AGRP (agouti-related protein) neurons in the hypothalamus, while leptin has the opposite effect (7,8). Ghrelin is able to increase GH release both in animals and in men, and it also induces PRL, ACTH, cortisol and aldosterone secretion *in vivo* (5,6,13,14). Ghrelin causes a slight increase in glucose levels and a reduction of circulating insulin (14). The discovery of ghrelin reinforced the concept of a third pathway of GH regulation (2,3,7,8). However, the physiological role of this potent endogenous GH-releasing peptide remains to be elucidated.

GHS and Ghrelin: mechanism of action and possible physiological role on GH release

GHS and ghrelin act both at hypothalamic and pituitary levels to modulate GH secretion (for reviews see ref. 3,7,8). These peptides stimulate the GHS-R in pituitary cells *in vitro* to induce GH release (5). When GHRH is associated to GHS or ghrelin *in vitro*, an additive response is observed in most studies. However, when these peptides are administered together with GHRH *in vivo*, a synergistic effect on GH release is observed. This indicates that GHS and GHRH act through different mechanisms and suggests a main hypothalamic site of action of GHS (6,14-16). This is confirmed by the lack of GH release after GHRP-6 or ghrelin in hypothalamic pituitary disconnection, both in animals and in men (17,18). It has been shown that an intact GHRH system is necessary for these actions to occur. Both GH pulsatility and GH responsiveness to ghrelin and GHS are decreased by the administration of antibodies against GHRH in rats (19). GHS-induced GH release is also blunted by a GHRH antagonist (20). GHS are not able to increase GH release in the *lit/lit* mouse, which has a GHRH receptor mutation, but they enhance hypothalamic *c-fos* expression, which is a marker of neuronal activity (21). GH response to GHS is inhibited in humans with GHRH receptor mutations, but the ACTH and PRL releasing effects are maintained, which suggests that the latter actions are mediated by the hypothalamus (22). The arcuate nucleus is the main target of ghrelin action, where it may bind and activate the GHS-R. GHS and ghrelin act centrally increasing electrical activity and *c-fos* expression in a subpopulation of cells in the arcuate nucleus, some of which are GHRH producing neurons (21). Moreover, one fourth of these GHRH neurons express the GHS-R, suggesting a direct effect of GHS in these cells (19). Ghrelin increases GHRH release from hypothalamic tissue *in vitro,* but this was not observed with GHS (23,24). It has also been shown that GHS increase GHRH release into the pituitary portal system in sheep (25). GHS and ghrelin do not influence hypothalamic somatostatin release in most studies, both *in vivo* and *in vitro* (23-25). However, GHS act as functional somatostatin antagonists (19,26). GHS increase the number of cells secreting GH and cause depolarization of the somatotroph, while somatostatin has opposite effects (26). Therefore, a model of action of GHS/ghrelin has been suggested, which would involve: 1) activation of GHRH producing neurons in the arcuate nucleus, with an increase in GHRH release; 2) amplification of the effect of GHRH at the somatotroph; 3) functional antagonism of somatostatin (2). GHS/ghrelin and GHRH bind to different pituitary receptors, and there is cross-talk between these receptors (for review see ref. 7). These peptides also stimulate different intracellular transduction pathways at the somatotroph. GHRH activates intracellular cyclic AMP and protein kinase A (PKA), while GHRP-6 stimulates protein kinase C (PKC), via inositol triphosphate signal transduction, with increase in intracellular calcium concentrations (figure 1) (2,4). Interestingly, it has been recently shown that ghrelin activates multiple, interdependent, intracellular pathways in porcine somatotrophs, involving PKA, PKC and extracellular calcium systems. This effect is broader than the action of

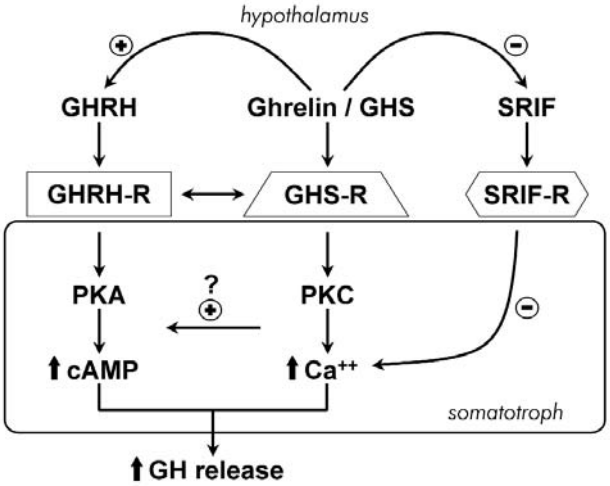

Figure 1. Schematic model of the possible interactions between GHRH, ghrelin/GHS and SRIF at hypothalamic and pituitary level.

most GHS, but similar to that described for GHRP-2 in this species (27). These results reinforce the possibility of cross-talk between these transduction pathways. However, the physiological role of these potent GH stimulators is still unclear. Controversial issues are how the hypothalamic peptide participates in GH modulation and whether circulating ghrelin has a role on pituitary GH secretion. In the rat ghrelin secretion occurs in a pulsatile manner, but has no correlation with GH pulses and is associated to food and sleep cycles (28). Circulating ghrelin levels are similar during GH peak and trough periods in the rat (29). Ghrelin immunoneutralization does not interfere with GH pulsatility, while GHRH antibodies block endogenous pulsatile GH release (30). In humans GHRH antagonist administration strongly inhibits 24h GH secretion, but fails to affect circulating ghrelin levels (31). However, in rats, intracerebroventricular or peripheral administration of GHSR-1A antagonists attenuates spontaneous GH secretion, due to a decrease in pulse amplitude and mean GH levels (32-34). Interestingly, a missense mutation in the GHS receptor, which severely impaired ghrelin binding, was associated with a case of familial short stature (35). It has also been shown in healthy volunteers that circulating ghrelin is related to GH pulses, suggesting that ghrelin participates in the pulsatile regulation of GH secretion or that the two hormones are regulated in parallel (36). Therefore, endogenous ghrelin might amplify the basic pattern of GH secretion (32-34). This peptide may also have a physiological role in GH release by optimizing somatotroph responsiveness to GHRH (37). Nevertheless, recent studies with ghrelin knockout animals failed to show a major effect on GH regulation (38). In contrast to predictions, these animals were not anorexic dwarfs (38). However, in transgenic models with decreased GHS-R mRNA expression in the arcuate nucleus, reduced GH and IGF-I levels were observed (39). Also, GHS-R knockout mouse had lower body weight and IGF-I values (40). These effects were only moderate, which is intriguing, as these peptides are quite potent GH stimulators. It has been previously suggested that the role of ghrelin on GH secretion might become more relevant during states of negative energy balance (30). However, further studies will be necessary to elucidate the physiological role of these peptides on GH secretion.

Modulation of GH release by GHRP-6 and Ghrelin in man

GHRP-6 and ghrelin increase GH release in a dose dependent manner, both *in vivo* and *in vitro* in several species, including man (1,5,6,13,16). The GH releasing activity of ghrelin is similar to that of GHRH *in vitro* (5). However, *iv* ghrelin administration at a dose of 1 μg/kg increases GH release potently in man, and this response is higher than that obtained with GHRH, hexarelin and GHRP-6 (13,14,41). This effect is not specific as an increase in PRL, ACTH, cortisol and aldosterone levels is also observed (14). Glucose levels increase and insulin values decrease after *iv* administration of this peptide (14). These latter effects and the aldosterone stimulation are not seen with other GHS. When ghrelin or GHRP-6 are administered together with GHRH a synergistic effect is seen, but this is better observed with injections of ghrelin at low doses (0,08 and 0,2 μg/kg) (14,16). The administration of GHRP-6 together with GHRH is an excellent test to diagnose GH deficiency in adults, but its usefulness in children is less clear (42). There is a highly reproducible response for GHS in normal subjects, studied in different occasions, differently than that observed for GHRH. There are no gender differences in the GH response to GHRP-6 and ghrelin (3,15,43), but an age related decrease of responsiveness has been reported for both peptides (3,15,43). It has been shown that obese subjects have blunted GH responses to GHRP-6 and ghrelin (44,45). Ghrelin-induced GH release is decreased by 55% in women with visceral adiposity and BMI of 362 kg/m^2 (45). Hyperglycemia, free fatty acids and somatostatin decrease GHRP-6 and ghrelin-induced GH response (46,47). Arginine was not able to alter GH responsiveness to ghrelin (48). The effect of cholinergic ago-

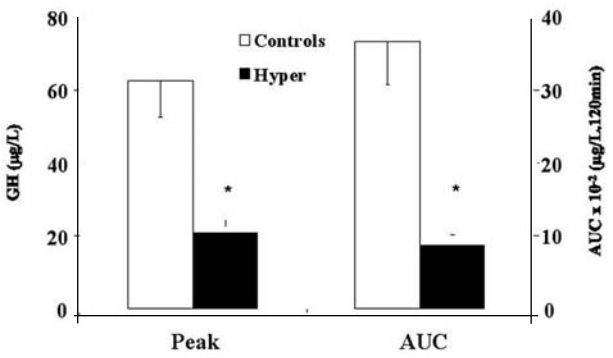

Figure 2. Mean GH peak and area under the curve (AUC) values after ghrelin administration in 6 hyperthyroid patients and 8 controls (mean ± SE; * *P*< 0,05) (from ref. 58).

nists and antagonists on ghrelin-induced GH release is controversial. Piridostigmine failed to modify GH responsiveness to both ghrelin and GHRP-6 (15,48). However, atropine blunted this response, but pirenzepine, a muscarinic receptor antagonist, was unable to alter GH release after ghrelin (49). These latter compounds only blunt GH response to GHRP-6, while they completely abolish GH response to GHRH (15). GH response to GHS was only attenuated by glucocorticoids and GH administration, which probably enhance hypothalamic somatostatin release (50,51). In patients with Cushing's disease a blunted GH response to both GHRP-6 and ghrelin has been reported by us and by others (52-55). In these patients GHS and ghrelin-induced ACTH and cortisol release is increased (54,55). These latter effects could be due to a direct action of these peptides at GHS-R in the corticotroph adenoma or, alternatively, activation of hypothalamic AVP and probably, to a lesser extent, of CRH pathways (7). Interestingly, chronic glucocorticoid administration does not interfere with GHRP-6 induced GH release (53). It has been previously suggested that the time of exposure to hypercortisolism is important for the GH response to these peptides. In patients with adrenal insufficiency a 72 h withdrawal of glucocorticoid replacement therapy does not influence the GH responsiveness to GHRP-6 (57). We have shown that in hyperthyroidism there is a decrease in GH responsiveness to GHRH while GHRP-6-induced GH release is maintained, which could suggest that thyroid hormones interfere mainly with GHRH-releasing mechanisms (58). However, we have recently shown that there is a decrease in the GH response to ghrelin in these patients, suggesting that thyroid hormones might interfere with additional pathways of GH release activated by ghrelin (figure 2) (59). In type 1 diabetes mellitus GH response to GHRP-6 and hexarelin is either normal or enhanced, demonstrating that hyperglycemia is unable to decrease GH release induced by these peptides, differently than in normal subjects (60). It has recently been shown that ghrelin-induced GH release is decreased in anorexia nervosa, which is an unexpected finding as these patients have high GH levels and enhanced responses to GHRH and GHS (61). GH-releasing compounds could represent an alternative treatment in GH deficient states. However, these substances have failed to show benefit over GH therapy, despite the fact that they are more physiological, as they induce endogenous pulsatile GH release.

CONCLUSION

Ghrelin is a novel hormone secreted from the stomach to the circulation. This peptide is also produced in the hypothalamus and other tissues, with both endocrine and paracrine effects. The acyl modification of its molecule is essential for enhancement of GH release and stimulation of food intake. Several questions remain to be answered concerning the roles of circulating and hypothalamic ghrelin on GH release. Ghrelin might have a physiological role on pulsatile GH secretion, but further studies are necessary to clarify its precise role on GH modulation.

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