
MINIREVIEW

Ghrelin - a New Endogenous Growth Hormone Secretagogue

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Summary

Ghrelin is a new endogenous peptide, discovered in 1999 by Kojima *et al.*, as the result of a search for an endogenous ligand for an orphan receptor of known structure and function. Ghrelin is composed of 28 amino acids and is produced mostly by cells of the stomach, hypothalamus, and hypophysis, but it has also been detected in other tissues. Its discovery is related to the development of a new hypothesis regarding the regulation of growth hormone secretion. It is an antagonist of somatostatin. Ghrelin activates the release of growth hormone from the somatotrophic cells of the hypophysis. It participates in the regulation of energy homeostasis, increases food intake, decreases energy output and exerts a lipogenetic effect. Its metabolic effects do not depend on the GH/IGF-I system, but are mediated by the NPY/Y1 and AGRP receptor system. Ghrelin influences the secretion and motility of the gastrointestinal tract, especially the stomach. The presence of ghrelin and its receptors has also been demonstrated in many other tissues. Its function in these tissues has not yet been studied, thus providing many possibilities for further research.

Key words

Energy expenditure • Ghrelin • Growth hormone secretagogue • Leptin • Ob/Ob Mice

Introduction

Recently, great significance has been associated with a new class of synthetic substances, so-called growth hormone secretagogues (GHS), which play an important role in the secretion of GH. Bowers and Momany discovered these substances in 1976 when working with analogues of metenkephalin (Bowers *et al.* 1977). It was found that these analogues are capable of releasing GH from primary cell cultures of the rat hypophysis. These are substances of peptide and non-peptide character (Maruna and Krijt 1995, Jenšovský *et al.* 2000). GHS

influence the secretion of growth hormone differently from GHRH. The GHS act at the level of the hypophysis and hypothalamus, but can exert effects in other parts of the central and peripheral nervous systems (see below, e.g. influence on gastric motility). Receptors with a high specificity for GHS have been detected. These receptors are associated with the G-protein and their activation leads to the release of calcium from the endoplasmic reticulum into the cell cytoplasm and depolarization of the cell membrane (Jenšovský *et al.* 2000). Using immunohistochemical methods and methods of *in situ* hybridization, these receptors were detected in many

tissues (myocardium, adrenal glands, gonads, liver, skeletal muscle, etc.) including the CNS (Papotti *et al.* 2000, Arvat *et al.* 2001). This points to the great variety in the functions of GHS, about which we know very little. Both endocrine and non-endocrine subtypes of this receptor, which may participate in modulating the biological activity of GHS, were found in the CNS and in peripheral organs (Ghigo *et al.* 2001).

Discovery of an endogenous ligand for GHS receptor

Until recently, only synthetic ligands for the above-mentioned receptor existed, but there were no known endogenous ligands. An “intracellular calcium influx assay” was used for the detection of a hypothetical ligand, during changes in intracellular concentrations of calcium were evaluated in response to various substances. Synthetically cultured cells expressing rat GHS-R were used. Various tissue extracts were added to such cell culture and changes in intracellular concentrations of calcium were estimated for extracts from the rat brain, lungs, heart, kidneys, stomach, and intestines. The greatest change in intracellular calcium concentration was found after adding the stomach extract. With further purification two types of peptides were obtained, which Kojima *et al.* (1999) named ghrelin and des-14-Gln ghrelin (the name comes for ghre, which is of Proto-Indo-European origin, meaning growth). Ghrelin is a peptide composed of 28 amino acids, differing from des-14-Gln ghrelin by the presence of glutamine in position 14. The presence of an octanoyl group on the hydroxy group of serine in position 3 is characteristic of the ghrelin molecule. In the organism, this peptide modification is unique. It is a post-translational modification, which is most likely essential for the function of this peptide in the organism (Bednarek *et al.* 2000).

With further detailed analysis, precursor molecules for both forms of the peptide were found: preproghrelin (117 amino acids) and prepro-des-14-Gln ghrelin (116 amino acids). Both ghrelin and des-14-Gln ghrelin are endogenous ligands for the GHS-receptor. Both forms of the peptide are products of one gene, but des-14-Gln ghrelin forms an alternate splicing of the ghrelin gene in the frame of post-transcriptional modification. This splicing is unique because it occurs in the peptide-coding portion of DNA, the exon. Normally, during alternate splicing, individual introns are removed, eventually with complete exons (Hosoda *et al.* 2000b).

The third known form of ghrelin is des-acyl-ghrelin, which lacks the hydrophobic substitution in the

amino acid chain. This could be a degradation product of the ghrelin molecule (Hosoda *et al.* 2000a).

Human ghrelin

With the help of rat cDNA coding for ghrelin, it has been possible to find a corresponding gene in the human genome. The peptide coding this gene differs from rat ghrelin in only two amino acids. It also consists of 28 amino acids with an octanoyl group on serine at the third position (Kojima *et al.* 1999). Histochemical and *in situ* hybridization studies demonstrated the presence of ghrelin in various tissues of the human organism: in the arcuate nucleus of the hypothalamus, in the hypophysis, with clearly highest incidence in the gastric tissue. Endocrine cells of the gastrointestinal tract are a main source of ghrelin in this localization. With the help of *in situ* hybridization, immunohistochemistry, and electron microscopy, the cells responsible for ghrelin production have been identified. These cells, originally called X/A-like cells, now ghrelin cells or Gr cells, represent a relatively large population of cells (20 %) localized primarily in the gastric mucosa, less in the pylorus and small intestine (Date *et al.* 2000b).

Regulation of ghrelin secretion

Gastric ghrelin and its secretion can be regulated by local or central stimuli. These concern mechanical stimuli, the action of digestive products on the gastric lumen, substances in the systemic circulation, or signals from the CNS. The exact mechanism of regulation is unknown. It has experimentally been found that simple expansion of the stomach does not lead to ghrelin secretion. The administration of a glucose solution is a strong stimulus.

Ghrelin as a new stimulator of growth hormone secretion

The growth hormone is a peptide hormone influencing growth and development of the organism. It is known that two hypothalamic hormones regulate hypophyseal secretion of the growth hormone: growth hormone releasing hormone (GHRH) and the inhibiting hormone, somatostatin.

The stimulating effect of ghrelin on GH production was demonstrated *in vitro* on a pituitary cell culture (Kojima *et al.* 1999). Afterwards, this was also demonstrated in rat models and later in human volunteers. In rats, both peripheral and intracerebral administration of ghrelin led to the stimulation of GH secretion from somatotrophic cells of the hypophysis (Date *et al.* 2000a, Tolle *et al.* 2001). The maximum plasma concentration of

GH was attained 15-20 min after the administration, with normalization within 60 min. However, increased expression of mRNA in the somatotrophic cells of the hypophysis was not observed (Date *et al.* 2000a). Repeated or continuous administration led to only a transient increase in plasma GH concentration. This could be caused by desensitization that is observed in other receptor systems. In this case, it was found that the decreased availability of receptors on the cell surface was caused by their decreased synthesis (Tolle *et al.* 2001).

Stimulation of GH secretion was also observed in humans after peripheral administration of synthetic ghrelin. The maximum concentration of GH was measured after 30 min, a return to basal values was reached within 180 min. From these experiments it follows that ghrelin is a strong stimulator of growth hormone secretion. Its administration leads to a dose-dependent stimulation of GH secretion. Its effect is several fold stronger than the effect of GHRH (0.2 µg/kg of ghrelin is equivalent to 1.0 µg/kg GHRH) (Takaya *et al.* 2000, Peino *et al.* 2000, Arvat *et al.* 2000).

GHRH is essential for full expression of the effect of ghrelin on GH stimulation. Simultaneous administration of GHRH and ghrelin synergistically leads to increased GH production. When GHRH antiserum was administered, there was no significant increase in GH due to ghrelin, as was the case when there was a defect in the GHRH receptor. GHRH stimulates secretion as well as synthesis of GH, whereas ghrelin has an antagonistic effect to somatostatin; this means that it only leads to stimulation of GH secretion (Takaya *et al.* 2000).

Regulation of secretion of other hypophyseal hormones

In experiments on rats (Kojima *et al.* 1999, Date *et al.* 2000a,b) and on human volunteers (Takaya *et al.* 2000, Peino *et al.* 2000, Arvat *et al.* 2001), the influence of intracerebral and peripherally administered ghrelin on other trophic functions of the hypophysis was also observed. There was a slight increase in plasma cortisol concentration and ACTH, and a slight increase in prolactin concentration, but there was no significant increase in LH, FSH, and TSH. The mechanism by which ghrelin influences the secretion of ACTH and prolactin is as yet unclear. It could be the result of an influence at the level of hypothalamus or hypophysis.

Influence of food intake and energy balance

A possible influence of synthetically prepared ghrelin and GHS on the regulation of energy homeostasis and food intake has been observed experimentally.

Changes in the regulation of food intake after peripheral and intracerebral administration of synthetic ghrelin in rats was studied. It was found that repeated or continuous administration led to a dose-dependent increase in food intake with the corresponding increase in body mass. Analysis of body composition documented a significant increase especially of fatty tissue, whereas there was no increase in bone and cartilage mass. No stimulation of growth was observed, although an increased respiratory quotient and decreased energy expenditure was found (Asakawa *et al.* 2001). The respiratory quotient increases with the preferential utilization of saccharides. This is in agreement with the described weight gain, which is primarily due to increase of fatty tissue, whose utilization is suppressed (Wren *et al.* 2000, Nakazato *et al.* 2001).

Changes in the plasma concentration of ghrelin in fasting rats, and the influence of realimentation were reported (Toshinai *et al.* 2001). After 48-hour fasting, there was increased expression of ghrelin mRNA in the stomach, but a decrease in the amount of actual peptides in gastric cells. Its plasma concentration was increased. After refeeding, there was a return to the original state, a decrease in plasma concentration of ghrelin and normalization of its concentration in cells of the gastric fundus. These changes indicate that, during fasting, the synthesis of ghrelin is stimulated and especially its secretion from the gastric endocrine cells is potentiated. This decreases the concentration of ghrelin in the endocrine cells and increases its levels in plasma. After realimentation, there is a return to normal values. The orexigenic effect of ghrelin has been compared to the effects of other known orexigenic peptides. Ghrelin has been shown to be the second strongest orexigenic peptide after neuropeptide Y (NPY). An attempt has been made to find the mechanism by which the effects of ghrelin could be explained. It seems unlikely that the metabolic effects of ghrelin are mediated by growth hormone. The influence of GH and ghrelin energy homeostasis was compared experimentally. The effect of ghrelin, independently of GH, on the regulation of energy homeostasis was demonstrated in GH-deficient rats. After repeated administration of ghrelin, the same changes in metabolism were observed as in rats without this deficit (Tschop *et al.* 2000).

The observation that ghrelin has an almost comparable effect to NPY led to the hypothesis that its action could be mediated by this peptide (Hewson *et al.* 2000). NPY is the key component of regulatory mechanisms of energy homeostasis, it stimulates food

intake and decreases energy output. NPY is produced by the neurons of the hypothalamic nucleus arcuatus. These neurons also express GHS receptors on their surface. The administration of antagonists of Y1 and Y5 receptors for NPY or administration of anti-NPY antibody decreased ghrelin-induced food intake and increased the body mass. Administration of ghrelin antibody suppressed its function, but had no influence on the effect on NPY in the regulation of food intake and energy homeostasis (Nakazato *et al.* 2001).

In other experiments, the relationship between ghrelin as an orexigenic peptide and an anorexigenic leptin was studied in Ob/Ob mice. Leptin is a peptide hormone produced primarily by adipocytes. Its serum concentrations are dependent on the amount of subcutaneous fat. The anorexigenic effect of leptin is mediated by inhibition of the synthesis and secretion of NPY. This suppresses appetite and increases energy output. Ob/Ob mice are defective in the gene for leptin, and are typically hyperphagic, obese, and sterile. A significantly higher expression of ghrelin in gastric cells and higher plasma concentrations were found in these animals in comparison to healthy mice. After administration of leptin, the concentration of ghrelin decreased, and reduced food intake and increased energy expenditure were observed. A significant decrease in ghrelin concentration after leptin administration was also observed even in lean mice without a defect in the leptin gene. It seems that the effect of both peptides is antagonistic, being mediated by the same NPY-Y1 receptor system (Shintani *et al.* 2001).

The gastrointestinal system

Another factor, possibly mediating the effects of ghrelin on the regulation of energy homeostasis, is its influence on gastric secretion and motility. Motility of the gastrointestinal tract plays a significant part in the regulation of energy homeostasis. Faster gastric emptying is related to an increased food intake and obesity, while slower gastric emptying is related to anorexia and cachexia. After peripheral and central administration of ghrelin in rats, it was found that there is a dose-dependent increase in gastric secretion and motility. Firstly, there is stimulation of gastric motility and, after a delay, there is increased secretion of HCl and pepsin. This order of events is very similar to the changes that occur after parasympathetic stimulation (vagal nerve). After cervical vagotomy or after administration of atropine (parasympatholytic), the effect of ghrelin on gastric

function was completely abolished. Thus, the effect of ghrelin on gastric function is mediated by the parasympathetic system. This was demonstrated by observing the changes in the expression of Fos protein in the hypothalamic nucleus tractus solitarius and the dorsomedial nucleus (the vagal centers) which participate in regulating gastric secretion and motility (Masuda *et al.* 2000, Asakawa *et al.* 2001).

The kidneys

Expression of the ghrelin gene was demonstrated in renal podocytes, mesangial cells, glomerular cells, and fibroblast-like cells. The presence of GHS-R on these cells was also demonstrated. It therefore seems that ghrelin is also produced in the kidneys where it can exert its paracrine/autocrine activity. Its relation to GH regulation could contribute to a new perspective of the pathophysiology of certain renal diseases. It is known that GH, whether independently or through IGF-1, increases renal perfusion, glomerular filtration, and tubular resorption of phosphates and sodium. Its effect has been associated with compensatory hypertrophy after nephrectomy or after hypertrophy in diabetes mellitus (Mori *et al.* 2000).

Hemodynamic effects

The presence of ghrelin receptors was observed in blood vessels including the aorta, the left ventricle, and the left atrium in rats. In human volunteers, the influence of ghrelin on hemodynamics was also studied. It was found that after its intravenous administration, there was a decrease in the mean arterial pressure (by 12 mm Hg), an increase in the cardiac index (by 16 %), and an increase in the pulse volume (by 22 %) (Nagaya *et al.* 2001).

Placenta

Similarly to the other components of the somatotrophic axis, ghrelin was detected in the human and also in the rat placenta. The highest concentration was attained in the first trimester, its concentration gradually decreased with the evolving pregnancy and tissue differentiation. Ghrelin was detected only in the cells of the cytotrophoblast. Its function in the placental tissue is not clear. It is possible that ghrelin takes part in the regulation of growth and differentiation of placental tissue, and could contribute to the regulation of GH secretion in the mother, fetus, or both simultaneously during pregnancy (Gualillo *et al.* 2001).

Conclusion

Ghrelin is a new endogenous peptide discovered during the search for an unknown endogenous ligand of a receptor of known structure and function. It is composed of 28 amino acids with a single octanoyl modification on the hydroxy group of serine at position 3, which is essential for its function. It is produced by endocrine cells of the stomach and hypothalamus, to a lesser extent also in several other tissues. This shows the wide-ranging functions of ghrelin, most of which are yet unclear, thus giving great possibilities for further research.

Ghrelin is a strong stimulator of GH secretion. Its discovery has brought a new perspective to the regulation of GH secretion. This physiological model includes GHRH, somatostatin, and ghrelin. GHRH is important for the growth and development of somatotrophic cells, and stimulates the synthesis and secretion of GH. The effect of GHRH is mediated by a receptor whose activation leads to the release of cAMP.

Regulation of secretion (not synthesis) is dependent on the antagonistic actions of ghrelin and somatostatin. Somatostatin is a hormone suppressing the release of GH from the somatotrophic cells of the hypophysis. Binding to the somatostatin receptor leads to the inhibition of adenylcyclase, the decrease of cAMP, and a decrease in intracellular calcium levels. The presence of all three components is important for proper regulation. Their combined action is as yet the strongest stimulation of GH secretion in humans.

Ghrelin is a significant orexigenic peptide. It causes increased food intake and decreased energy expenditure and has a lipogenetic effect. This positive metabolic balance is one of the main assumptions of

growth of the organism and is thus essential for the effect of GH. Ghrelin could be a peripheral signal informing the CNS of food intake and of the nutritional status. On the basis of this information, the activation or inhibition of secretion of the growth hormone and growth of the organism could follow. It is possible that ghrelin is a new link between the GH/IGF-1 axis and the neuroendocrine regulation of energy balance.

The discovery of ghrelin and its relation to secretion brings new clinical possibilities to the pathophysiology, diagnosis, and therapy of endocrine disorders, especially of GH deficiency. Recently, several studies have appeared indicating that decreased secretion of GH can possibly be primarily caused by aberrant synthesis and secretion of ghrelin. Ghrelin and its simultaneous administration with GHRH could become a new diagnostic test for distinguishing GH deficits. It could become the "gold standard" in determining GH reserves in adults, and distinguishing subjects with adult GH deficiency (Baldelli *et al.* 2001, Arvat *et al.* 2001). It could be used in the therapy of diseases associated with decreased GH production.

On the basis of the above-mentioned effects of ghrelin, it could be used in patients in catabolic states associated with sepsis, surgery, during administration of corticoids, in cancer (in conditions associated with the reduction of non-fatty tissue and with anorexia). It could be used in the treatment of obesity, by blocking its effect, or in anorexia and cachexia.

As yet there is little information about ghrelin. To increase this knowledge, other studies will be necessary to extend a wide range of possibilities concerning physiological implications of this substance.

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