ENDOCRINE AND METABOLIC ACTIVITIES OF A RECENTLY ISOLATED PEPTIDE HORMONE GHRELIN, AN ENDOGENOUS LIGAND OF THE GROWTH HORMONE SECRETAGOGUE RECEPTOR

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Ghrelin is a member of the group of growth hormone secretagogues (GHSs). It is a peptide hormone, recently isolated from stomach as an endogenous ligand for the growth hormone secretagogue receptor (GHS-R). Ghrelin is mostly produced by the stomach, although its production has been proved in various tissues. It is a potent releaser of growth hormone (GH) from anterior pituitary cells, but it also stimulates the release of other hypophyseal hormones. Ghrelin stimulates food intake and induces metabolic changes leading to an increase in body weight and body fat mass. This effect seems to be independent of GH action and needs an intact NPY/AGRP (neuropeptide Y/agouti-related protein) system. Plasma ghrelin levels are decreased in obesity, elevated in cachexia and show a diurnal rhythm. Its preprandial elevation suggests, that it might be a signal for meal initiation. Ghrelin further stimulates the release of gastric acid and gastric motility and affects pancreatic functions. It has vasodilatatory, cardioprotective and antiproliferative effects. This article is focused on ghrelin's endocrine and metabolic functions.

Key words: Ghrelin - Des-acyl ghrelin - Growth hormone GHRH Metabolism - Nutrition

The history of GHSs dates back to 1977, when Bowers developed a group of small peptides which stimulated the release of GH from anterior pituitary cells in vitro (BOWERS et al. 1977). Further studies led to the development of several more potent peptides, with a strong GH stimulatory effect in vitro and in vivo (BOWERS et al. 1984). However in 1982 growth hormone-releasing hormone (GHRH) was characterised. It was postulated, that GH secretion by the anterior pituitary gland was regulated by an interaction of two hypothalamic hormones – GHRH and somatostatin. This approach however could not explain the effects of GHSs, whose mechanism of action on GH release is different. The effects of GHRH and GHSs on GH release are complementary, their simultanous administration is the most potent GH releaser to date. This hypothesis was supported by the identification of different receptors and intracellular signalling (ARVAT et al. 2001; BALDELLI et al. 2001).

Isolation of the growth hormone secretagogue receptor. Later a GHS-R DNA was isolated (HOWARD et al. 1996). GHS-R is a classical G-protein coupled receptor. Two types of GHS-R DNAs (cDNAs) have so far been identified and designated receptor 1a and 1b. Unlike the GHS-R 1a, the GHS-R 1b fails to bind GHSs and its function remains to be elucidated (MCKEE et al. 1997).

There is strong evidence suggesting, that there are several additional subtypes of the GHS-R, which modulate a variety of endocrine and non-endocrine activities of GHSs. Binding sites, different from the GHS-R 1a and 1b have been found in a wide range of tissues (PAPOTTI et al. 2000; GHIGO et al. 2000). The GHS-R 1a is widely present in various tissues. It was found in the anterior pituitary gland, hypothalamus, stomach, intestine, pancreas, myocardium, aorta, adrenal, lung, liver, skeletal muscle, kidney, thyroid, adipose tissue, lymphatic gland, parathyroid, placenta, mammary gland,

prostatic gland, and spleen (Date et al. 2000; Nagaya et al. 2001; Papotti et al. 2000; Shuto et al. 2001).

Endogenous ligand. Despite intensive research, the isolation of an endogenous ligand of the GHS-R remained elusive until recently. In 1999 Kojima isolated the endogenous ligand, which was later named ghrelin, from a rat stomach (Kojima et al. 1999). Ghrelin is predominantly produced by the stomach, with substantially lower amounts derived from the bowel, pancreas, kidney, placenta, pituitary and hypothalamus (Date et al. 2000a; Korbonits et al. 2001; Kršek et al. 2002; Volante et al. 2002; Wierup et al. 2002). In stomach it is present in endocrine cells, which account for about 20% of the endocrine cell population in oxyntic glands. Ghrelin cells have no continuity with the lumen, but they are closely associated with the capillary network running through the lamina propria. Ghrelin cells seem not to operate under gastrin control (Dornonville de la Cour et al. 2001). Removal of the acid-producing part of the stomach in rats reduces serum ghrelin concentration by cca 80%, which supports the view, that the main source of ghrelin is the stomach (Date et al. 2000a).

Structure of ghrelin. Ghrelin is a peptide containing 28 amino acids, in which the serine 3 residue is n-octanoylated. This posttranslational peptide modification had not been previously observed and it is of a critical importance for its binding to GHS-R 1a, its GH–releasing and other endocrine activities, as well as for its transport over the blood-brain barrier. Human and rat ghrelin differ in 2 amino acids, in positions 11 and 12 (Bednarek et al. 2000; Kojima et al. 1999; Matsumoto et al. 2001; Muccioli et al. 2001).

Molecular variants of ghrelin. Later studies led to the isolations of another natural endogenous ligands of the GHS-R. One of them is des-Gln^{14}–ghrelin, a 27-amino acid peptide, where there is glutamin missing in the 14th position. Des-Gln^{14}–ghrelin possesses the same biologic activities as ghrelin (Hosoda et al. 2000a).

Des-acyl ghrelin, which lacks a hydrofobic chain substitution on position 3, is another molecular form of ghrelin. The ratio of these two forms in a rat´s stomach is 2:1 in favour of the desacylated form, which also predominates in systemic circulation in rats (Hosoda et al. 2000b) and humans (Yoshimoto et al. 2002). This form of ghrelin lacks ghrelin´s endocrine activities, however it is able to exert some non-endocrine actions (Baldanzi et al. 2001).

Secretion of ghrelin. Ghrelin plasma level decreases after food intake and increases during food deprivation in rats and humans (Asakawa et al. 2001; Toshinai et al. 2001; Tschöp et al. 2001). In fasting rats an increase in ghrelin mRNA together with a decrease in its content in the stomach and an increase in plasma ghrelin was observed. These changes were abolished after realimentation (Toshinai et al. 2001). In humans plasma ghrelin level is negatively correlated with BMI and percentage body fat content. Plasma ghrelin is decreased in obese patients and increases after weight reduction (Tschöp et al. 2001), its level is elevated in patients with anorexia nervosa and decreases after weight normalization (Becker et al. 1999, Otto et al. 2001).

Intravenous or peroral administration of glucose leads to the suppression of plasma ghrelin in rats as well as in humans (Ariyasu et al. 2002, Nakagawa et al. 2002; Shiiya et al. 2002). In hyperinsulinemic conditions, either causing hypoglycemia, or in euglycaemic clamp, plasma ghrelin concentrations are suppressed (Lucidi et al. 2002; McCowen et al. 2002; Saad et al. 2002). Based on these observations, insulin might directly or indirectly mediate the effect of nutrition or energy state on plasma ghrelin. The decline in plasma insulin with fasting would lead to an increase in plasma ghrelin, while post-prandial insulin release would suppress plasma ghrelin concentration. Similarly the hyperinsulinemia of obesity would suppress plasma ghrelin, while the lower insulin level in lean subjects would lead to increased ghrelin concentrations. Some studies report a reduction in insulin secretion in humans after ghrelin administration (Broglio et al. 2001). It could be a hormonal response to fasting, which maintains glucose level during fasting by inhibition of insulin secretion.

In contrast, another observation was made in rodents. The administration of insulin led to the stimulation of ghrelin secretion in the stomach (Toshinai et al. 2001). Whether interspecific or other mechanisms are involved needs to be further clarified.

Acromegalic patients show lower ghrelin levels than healthy controls, which are unaffected by oral glucose tolerance test. Low ghrelin levels might be caused by the putative negative feedback mechanism of GH and IGF-I, or the ghrelin levels might be supressed by hyperinsulinemia, as the lowest ghrelin levels were found in patients with the most severe insulin resistance (Cappielo et al. 2002).

Subjects with growth hormon deficiency (GHD) have lower ghrelin levels in comparison with healthy control subjects, which are not modified after 1 year of

GH replacement therapy, as observed by JANSSEN et al. (2001). This study included patients with idiopatic GHD, patients after hypophysectomy or radiotherapy. The explanation of this remains to be cleared. GHD patients had higher BMI and percentage body fat than the control group, which decrease after one year of GH therapy. So the supposed decrease in ghrelin plasma level due to a negative feedback of GH therapy, could be blunted by the increase in ghrelin level due to a reduction of adiposity. Also the effect of other pituitary hormone deficiency or chronic pituitary hormone replacement could play a role.

An infusion of somatostatin and its analogues causes a significant decrease in plasma ghrelin concentrations (BROGLIO et al. 2002b; NORRELUND et al. 2002). Glucose and lipid-heparin infusion, which trigger hypothalamic somatostatin release, reduces, but does not eliminate the GH response to ghrelin (BROGLIO et al. 2002), whereas GH response to GHRH is almost completely abolished by somatostatin(BROGLIO et al. 2002; DI VITO et al. 2002).

The infusion of GHRH in rats results in a significant increase in pituitary gene expression of ghrelin and its receptor system, suggesting that this system in the pituitary gland could modulate the regulation of growth hormone secretion by GHRH (KAMEGAI et al. 2001; YOSHIHARA et al. 2002).

Endocrine activities of ghrelin. Ghrelin is a dose-dependent stimulator of GH secretion in rats, in vivo and in vitro. Its stimulatory effect in vivo is much stronger than that of GHRH (KOJIMA et al. 1999; SEOANE et al. 2000). Concentrations of prolactin, ACTH, LH, TSH and leptin are not affected by its peripheral administration in rodents, however its intracerebroventricular (ICV) administration leads to stimulation of secretion of ACTH, and inhibition of secretion of TSH, with no change of LH or prolactin levels (DATE et al. 2000b; WREN et al. 2000). After intravenous administration in rats, ghrelin blocks the stimulated secretion of somatostatin, whereas its basal secretion remained unaffected (TOLLE et al. 2001).

In humans, an intravenous administration of ghrelin causes a dose-dependent increase in serum GH levels. In a comparison to GHRH, ghrelin is a stronger stimulator of GH release (ARVAT et al. 2000; GHIGO et al. 2001; PEINO et al. 2000; TAKAYA et al. 2000). Ghrelin administration leads to an increase in prolactin, ACTH and cortisol levels (ARVAT et al. 2001b). Serum levels of FSH, LH, TSH remain unaffected (TAKAYA et al. 2000). A stimulatory effect on aldosteron secretion in humans was observed (ARVAT et al. 2001a). Also in patients with isolated GHD ghrelin causes a significant increase in GH level. Its stimulatory effect is stronger than that of GHRH + arginine or insulin induced hypoglycemia, but GH level is lower than in healthy controls. Ghrelin in these patients increases levels of ACTH, cortisol and PRL, which indicates, that this endocrine activity is fully independent of mechanisms underlying the GH-releasing effect (AIMARETTI et al. 2002).

Although the presence of ghrelin-producing cells in hypothalamus has been reported, their number is small. It is questionable, if GH release is controlled by the ghrelin from the hypothalamus or from the stomach, which is the main source of circulating ghrelin. A blood-to-brain and brain-to-blood transport of acylated ghrelin has been observed, whereas non-acylated ghrelin entered the brain by diffusion (BANKS et al. 2002). After a repeated administration of ghrelin in rats, the GH level did not substantially increase, this occured only after a break of 3-4 hours. Spontaneous episodes of GH secretion were not observed during the 3-hour interval (TOLLE et al. 2001). Also no change in GH mRNA expression in anterior pituitary was observed. As GHRH stimulates GH synthesis and secretion at the same time and GHS-R is expressed in hypothalamic cells secreting GHRH, this is another supporting fact, that ghrelin needs an intact GHRH system for its stimulatory effect. After administration of GHRH antiserum in rats, or hypothalamo-pituitary disconnection, the GH response to GHSs is strongly inhibited (TANNENBAUM et al. 2001). Studies in persons with an inactivating defect of the GHRH receptor gene suggest, that in humans the amplitude of GH pulses is driven by GHRH and the timing of GH pulses is primarily supervised by oscillations in somatostatin and/or ghrelin concentrations (ROELFSEMA et al. 2001).

Central metabolic effects of ghrelin. Ghrelin after administration in rats leads to an increase in food intake together with a dose-dependent weight gain due to a significant increase in fat tissue with no change in lean body mass, the amount of bone tissue or stimulation of growth (NAKAZATO et al. 2001; WREN et al. 2000). Respiratory quotient increases, which means an increase in sacharide metabolism and a decrease in fat utilisation. The lipogenetic effect of ghrelin seems to be independent of GH action, as GH increases energy expenditure and causes a decrease in body fat mass with no change in respiratory quotient. This effect was confirmed in

GH-deficient mice, in which ghrelin also leads to weight gain (TSCHÖP et al. 2000).

Expression of GHS-R was found in neurons of nucleus arcuatus secreting NPY and AGRP. NPY is one of the most effective stimulators of food intake and weight gain. During ghrelin administration, the expression of markers of neuronal activation (Fos and Egr-1 protein) as well as the expression of NPY mRNA and NPY secretion increased in these neurons (NAKAZATO et al. 2001; SHINTANI et al. 2001). During administration of antibodies against NPY or antagonists for NPY-receptor Y1 and Y5, ghrelin-induced hyperfagia was inhibited. During ICV administration of antibodies against ghrelin no inhibitory effect on NPY induced hyperfagia was observed (NAKAZATO et al. 2001). Neurons producing NPY and AGRP colocalize in nucleus arcuatus, and AGRP antagonists abolish ghrelin induced feeding. This data suggest, that ghrelin uses the NPY/AGRP system for its effects (HORVATH et al. 2001).

Ghrelin blocks leptin-induced feeding reduction and similarly leptin suppresses ghrelin-induced feeding. These results indicate, that ghrelin might antagonize leptin action in the regulation of the NPY system (NAKAZATO et al. 2001).

Ghrelin also inhibits serotonin release from rat hypothalamic neuronal terminals in vitro, the same as orexin A and orexin B. This could also account for the feeding stimulatory effect of this peptide (BRUNETTI et al. 2002).

Eating disorders. Plasma ghrelin levels are negatively correlated with body mass index (BMI), body fat mass, adipocyte size, plasma insulin levels, plasma glucose levels and plasma leptin levels (TSCHÖP et al. 2001). Circulating ghrelin levels in normal-weight subjects show a diurnal profile similar to GH diurnal rhytm (SHIIYA et al. 2002). Plasma ghrelin concentrations rise progressively for one to two hours before each meal and fall to through levels within one to two hours after beginning of eating. In healthy volunteers administered ghrelin caused hunger sensations, so its increase could have a role in meal initiation. Between-meal ghrelin values rise gradually throughout the day in a diurnal pattern, with a nadir between 9 a.m. and 10 a.m. and a peak between midnight and 2 a.m. (CUMMINGS et al. 2002).

In obese individuals ghrelin levels are decreased, with no change after food intake (ENGLISH et al. 2002). This is a reversible condition, after weight loss the mean plasma ghrelin levels increase. There is a possitive correlation between the percentage decrease in body weight and BMI and the percentage increase in the area under the curve of ghrelin secretion (CUMMINGS et al. 2002). Obese patients also show marked impairment in spontaneous secretion of GH as well as in the somatotroph responsiveness to all provocative stimuli. GH insufficiency in obese patients has been reported reversible after long-term diet and marked weight loss. It is likely that alterations in the influence of ghrelin together with the alteration of the NPY/leptin interplay could have a role. Among metabolic alterations, the chronic elevation of free fatty acid levels and hyperinsulinism probably have a key role in causing GH insufficiency in obesity, associated with low ghrelin levels (MACCARIO et al. 2002).

In patients with anorexia nervosa plasma ghrelin levels are high (BECKER et al. 1999). After therapeutic intervention causing an increase in body weight, a significant decrease in circulating ghrelin levels was observed (OTTO et al. 2001). In bulimia nervosa, mean plasma ghrelin levels are significantly higher than those in the control group, which indicates a possible role of habitual binge eating and purging behavior on circulating ghrelin levels and a possible role of ghrelin inducing hyperphagia through the appetite control system in these patients (TANAKA et al. 2002).

The orexigenic effect of ghrelin might also play a crucial role in hyperfagia seen in Prader-Willi syndrome (PWS), which is characterized by excessive appetite and progressive massive obesity. Plasma ghrelin is high in PWS and a positive correlation was found between plasma ghrelin and subjective ratings of hunger in these subjects (DELPARIGI et al. 2002).

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