Influence of ghrelin on energy balance and endocrine physiology

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Abstract

Ghrelin is an endogenous ligand of the growth hormone secretagogue receptor. It is mainly secreted by stomach cells but has also been shown to be present in the hypothalamus, pituitary, gonads and many other organs. Ghrelin is a regulator of energy homeostasis and GH secretion. Many studies have been done examining the influence of ghrelin on different organs. Ghrelin may play an important role in pathophysiology of some endocrine diseases. The relationship between ghrelin and pituitary, gonads and thyroid function appears to be specially interesting.

INTRODUCTION

Ghrelin is an endogenous ligand of the receptor (GHS-R) activated by substances stimulating growth hormone (GH) secretion (GH secretagogues – GHSs). It was first identified and described by Kojima in 1999 as a 28-aminoacid peptide. Ghrelin was demonstrated to be present in two forms: acylated and nonacylated. Acylated ghrelin has undergone the process of acylation by n-octanoic acid at its Ser^3 residue (Kojima *et al.* 1999). Nonacylated ghrelin is a predominant form present in human serum and is not bind by any transporter. Acylated ghrelin constitutes about 10% of circulating peptide and is transported mostly by lipids (DeVriese *et al.* 2005; Hosoda *et al.* 2003). Ghrelin is inactivated by serum proteases and tissue esterases. Its half-life is 30 minutes.

Circulating ghrelin is predominantly produced and secreted by endocrine cells of the mucosa of the stomach (Date *et al.* 2000; Dornonville *et al.* 2001; Kagotani *et al.* 2001; Kojima *et al.* 1999). It was shown that after gastrectomy ghrelin secretion is reduced by approximately 65% (Ariayasu *et al.* 2001). It indicates that ghrelin is also secreted in other organs. Ghrelin synthesis and secretion was also demonstrated in small and large intestine, arcuate nucleus of the hypothalamus, pituitary, kidneys and placenta (Date *et al.* 2000; Gualillo *et al.* 2001; Kagotani *et al.* 2001; Kojima *et al.* 1999; Korbonits *et al.* 2001; Mori Y *et al.* 2000). There are also studies indicating the presence of ghrelin in testes, ovaries, thyroid gland, adrenal glands, liver, lungs, adipose tissue, pituitary tumours and carcinoid (Gnanapavan *et al.* 2002; Kanamoto *et al.* 2001; Ueberberg *et al.* 2008). It might be suggested that widely distributed ghrelin regulates different functions in the body.

Reference values of ghrelin concentration in human serum range from 130 to 260 pmol/l (Tschőp *et al.* 2000; Wasko *et al.* 2004). A negative correlation between the concentration of ghrelin

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and the age of examined subjects was demonstrated as well as the relationship between the concentration of ghrelin and gender (Rigamonti *et al.* 2002) Considering significant discrepancies in serum concentrations of ghrelin presented in different studies it is recommended to measure ghrelin levels also in control healthy subjects to evaluate whether serum ghrelin concentration in different disorders is statistically different from healthy individuals (Kosowicz *et al.* 2011).

Ghrelin GHS-receptor (GHS-R) was first cloned by Howard and co-workers (Howard *et al.* 1996). Two isoforms of the receptor have been identified: type 1a (366aa) and 1b (289aa – does not bind ghrelin). GHSR 1a consists of 366 aminoacids with seven transmembrane regions and is coupled with Gα11 protein. The binding of specific ligand to the receptor leads to the activation of phospholipase C signaling pathway followed by the separation of Gα11 subunit and the release of Ca^{2+} from intracellular stores (Herrington & Hille 1994; Howard *et al.* 1996; Petersenn *et al.* 2001). There are studies indicating the expression of GHS-R in the pituitary, hippocampus, pancreas, testes, thyroid, spleen, myocardium, adrenals, pituitary tumours, tumours type of insulinoma, gastrinoma and carcinoid, as well as in rat GH_3 cells (Gnanapavan *et al.* 2002; Papotti *et al.* 2000) The expression of GHS-R is most probably regulated by thyroid hormones, glucocorticosteroids and 17β estradiol (Guan *et al.* 1997; Kojima *et al.* 1999; Petersenn *et al.* 2001). The influence of 17β estradiol might explain the higher expression of GHS-R mRNA in female pituitaries compared with male glands.

It was first suspected that only acylated ghrelin has any biological activity (Bednarek *et al.* 2000) It is now known, however, that nonacylated ghrelin might exert significant biological actions. The mechanism of action of nonacylated ghrelin is not yet clear, however, this action is most probably not mediated by GHS-R 1a (Martini *et al.* 2006)

More studies with new information on the role of ghrelin in different biological processes in the body have been accumulated. The mechanisms of ghrelin influence on energy homeostasis and GH release have been well recognized. Other biological actions of ghrelin, both in endocrine and non-endocrine organs, are still intensely studied. The actions the authors of this summary are mostly interested in, because of the studies that they have been conducting, are discussed below.

GHRELIN AND ENERGY BALANCE

So far the best known function of ghrelin is the regulation of energy balance (Nakazato *et al.* 2001). Ghrelin regulates gastric acid secretion and bowel motility. Moreover, it transmits the signal to the hypothalamus about the current energy demand. Ghrelin strongly stimulates appetite and plays the main role in endocrine and metabolic response to fasting. Given intravenously it stimulates appetite in humans (Arvat *et al.* 2000). In rodents, intravenous administration of ghrelin results in obesity by increasing food intake and inhibiting fat utilization (Tschőp *et al.* 2000). Short-term peripheral or central stimulation by ghrelin results in the increase of appetite, whereas chronic ghrelin administration leads to increased food intake and inhibition of lipolysis and finally to positive energy balance (Horvath *et al.* 2002; Nakazato *et al.* 2001; Wren *et al.* 2001). The excess of ghrelin causes the accumulation of fat tissue and leads to abdominal obesity, which is not only the result of orexigenic actions of ghrelin, but also the GHS-R-dependent lipid retension (Davies *et al.* 2008) It was demonstrated that orexigenic and adipogenic effects of ghrelin are independent of its ability to stimulate GH secretion (Tschőp *et al.* 2000). Ghrelin is the first discovered peripheral hormone which directly acts in hypothalamic centres of appetite regulation. Ghrelin stimulates NPY and AGRP synthesis in arcuate nucleus of the hypothalamus leading to the increase of orexin and MCH production, which are hormones transmitting the signal of increased energy demand. Moreover, ghrelin inhibits the synthesis of α MSH, resulting in the inhibition of CRH and TRH production in the hypothalamus, and finally to the accumulation of fat tissue (Horvath *et al.* 2002; Nakazato *et al.* 2001; Tschőp *et al.* 2000). Leptin is considered to be ghrelin antagonist in this complex system. Protein kinase system activated by AMP (AMPK) functions as ghrelin signal transmitter in the cells of hypothalamus. Other ghrelin signal mediators are glicocorticosteroids, cannabinoids, leptin and insulin. Ghrelin and leptin are complementary components of one regulatory system which informs CNS about the energy balance. There are studies suggesting that ghrelin inhibits leptin secretion (Horvath *et al.* 2001; Shintani *et al.* 2001). It was demonstrated that serum ghrelin levels are increased shortly before the meal and might result in hunger and meal initiation. Serum ghrelin levels are increased in patients with anorexia nervosa and after fasting and decreased in obese subjects and after the meal (Ariayasu *et al.* 2001; Tschőp *et al.* 2000; Tschőp *et al.* 2001). It was shown that the decrease in serum ghrelin level after the meal is significantly lower in obese subjects compared with subjects with normal BMI, and, as a result, obese subjects achieve satiety later and after bigger meals. Briggs *et al.* (2010) demonstrated that obesity caused by hyperalimentation results in the resistance to ghrelin by decreasing the sensitivity of NPY/AGRP neurons to circulating ghrelin.

Peripheral metabolic actions of ghrelin include the inhibition of AMPK system in liver and adipose tissue leading to the increase of gluconeogenesis, fatty acid and triglycerides synthesis and fat tissue accumulation. Moreover, ghrelin reduces glucose-dependent insulin secretion, resulting in the increase of serum glucose levels and impaired glucose tolerance (Korbonits & Grossman 2004; Tong *et al.* 2010).

The increase of serum ghrelin level during fasting might result in the difficulties in maintaining the

reduced weight in subjects who have been on a diet. On the other hand, gastric bypass surgery as a treatment of obesity results in a significant ghrelin level decrease and helps maintain the reduced weight (Cummings *et al.* 2002).

GHRELIN AND PITUITARY

Ghrelin has a strong GH-releasing activity (Takaya *et al.* 2000). Its stimulatory effect is stronger that of GHRH (Hataya *et al.* 2001). It seems probable that ghrelin, together with GHRH and somatostatin, regulates GH secretion. It's been demonstrated that circulating ghrelin can directly stimulate GH release via GHS-R in the pituitary. Moreover, ghrelin was shown to increase *pit-1* expression, which influences GH gene expression (Garcia *et al.* 2000). Since ghrelin and its receptor have been identified both in pituitary and in hypothalamus (Gnanapavan *et al.* 2001; Kagotani *et al.* 2001; Korbonits *et al.* 2001a; Korbonits *et al.* 2001b), it can not be excluded that ghrelin acts in pituitary cells in autocrine and paracrine manner.

Ghrelin exerts stimulatory effect on PRL secretion (Arvat *et al.* 2001; Kojima *et al.* 2001) although there are studies demonstrating that ghrelin inhibits prolactin release in rodents during adolescence (Tena-Sampere *et al.* 2004). It's been hypothesized that the influence of ghrelin on PRL release is age dependent and is inhibitory before and stimulatory after puberty (Barreiro & Tena-Sampere 2004)

In hypothalamo-pituitary-adrenal axis ghrelin was demonstrated to stimulate ACTH release, probably indirectly, by increasing CRH secretion (Arvat *et al.* 2001; Kojima *et al.* 2001). In physiology this effect is less pronounced. Ghrelin inhibits pulsatile LH secretion in rats after ovariectomy treated with estradiol.

mRNA of ghrelin and GHS-R was found in normal pituitary and in pituitary tumours (Korbonits *et al.* 2001a; Korbonits *et al.* 2001b; Petersen *et al.* 2001; Skinner *et al.* 1998; Wasko *et al.* 2006). Expression of GHS-R was demonstrated in majority of somatotroph, lactotroph and corticotroph adenomas and very rarely in gonadotroph, thyreotroph and non-functioning adenomas (NFPA) (Korbonits *et al.* 2001b; Skinner *et al.* 1998). Kim *et al.* (2001) demonstrated a negative correlation between ghrelin levels and tumour size as well as the relationship between ghrelin concentration and the grade of the tumour in Hardy's classification. Ghrelin mRNA levels were highest in normal pituitary. In pituitary tumours, the highest ghrelin expression was found in NFPA, lower in somatotroph and gonadotroph and the lowest in PRL secreting adenomas. GHS-R expression was highest in somatotroph tumours, lower in NFPA, and the lowest in prolactinomas and gonadotropinomas. Korbonits *et al.* (2001b) found ghrelin mRNA to be present in normal pituitary as well as in somatotroph, corticotroph, lactotroph and gonadotroph adenomas and NFPA. The highest level of ghrelin mRNA expression was demonstrated in NFPA and then in somatotropinomas. Similar ghrelin expression was demonstrated in normal pituitary and prolactinomas, lower in gonadotroph tumours and the lowest in corticotroph adenomas. Moreover, Korbonits *et al.* (2001b) demonstrated the expression of GHS-R 1a and 1b in all above mentioned tissues. The expression of GHS-R 1a was highest in somatotroph adenomas, then lower in corticotroph and lactotroph adenomas, lower in normal pituitary cells and NFPA and almost absent in gonadotroph adenomas.

Ghrelin is considered to be an antiproliferative factor. It's been demonstrated that in vitro ghrelin inhibits the proliferation of neoplastic cells. Thus, the correlation between the size of the tumour and ghrelin concentration, described above, might be explained. The antiproliferative effect of both acylated and non-acylated ghrelin on neoplastic cells was demonstrated. In the cells of breast cancer, ghrelin expression was correlated with cells differentiation and was the highest in G1 grade and lowest in G3 grade. The available studies have demonstrated that ghrelin influences the proliferation of neoplastic cells acting through receptor other than GHS-R.

GHRELIN AND THYROID GLAND

The relationship between ghrelin and thyroid gland also seems to be very interesting. There are that have revealed the influence of thyroid hormones on ghrelin concentrations (Caminos *et al.* 2002). When the level of ghrelin mRNA was examined in stomach mucosa and in bloodstream of rats in which hyperthyroidism or hypothyroidism were induced, it was found to be decreased in hyperthyroidism and increased in hypothyroidism. Authors of the study assumed that thyroid hormones mediate energy balance via ghrelin. It was expected that in rats in which hyperthyroidism was induced, ghrelin levels would be increased to reduce the negative energy balance present in this condition. Furthermore, considering the decrease of appetite and the amount of ingested food in hypothyroid patients, it would seem reasonable to find lowered ghrelin concentrations. The above hypotheses were not confirmed and it was again hypothesized that hypothyroidism is connected with the resistance to appetite-stimulating effect of ghrelin. The attempts have been made for the past ten years to explain the relationship between ghrelin and the thyroid function. In most studies the decreased concentrations of ghrelin in hyperthyroidism were demonstrated (Giménez-Palop *et al.* 2005; Kosowicz *et al.* 2011; Riis *et al.* 2003; Tanda *et al.* 2009). The studies in hypothyroidism are much more contradictory. The concentrations of ghrelin were found to be both increased (Gjedde *et al.* 2008; Kosowicz *et al.* 2011) and decreased (Altinova *et al.* 2006) as well as comparable to control group (Giménez-Palop *et al.* 2005; Tanda *et al.* 2009). However, the contrary results

might be the effect of different criteria used to create the examined group.

The changes of ghrelin concentrations in thyroid disorders are most probably secondary to the changed metabolic state of the body. It might be assumed that it's not ghrelin that is responsible for the stimulation of appetite in hyperthyroid subjects, and its decreased secretion is the result of the negative feedback (Riis *et al.* 2003). Many studies have been considering thyreotoxicosis a catabolic state, similarly to anorexia or cancerous cachexia. Elevated ghrelin concentration and intensified catabolism could be what these conditions have in common (Gjedde *et al.* 2008; Riis *et al.* 2003; Somogyi *et al.* 2011). Thus, it must be considered that hypoghrelinemia in hyperthyroidism and hyperghrelinemia in hypothyroidism are the elements of compensatory mechanism. Valcavi *et al.* (1992) demonstrated that the concentrations of GH and IGF-1 were lowered in hypothyroid subjects. Low levels of GH and IGF-1 might lead to the compensatory increase of ghrelin levels in subjects with hypothyroidism.

It is known that subjects with thyreotoxicosis present with increased insulin concentrations and insulin resistance. The negative correlation between ghrelin and insulin were confirmed in many studies. Thus, hypoghrelinemia in hyperthyroid subjects might result from elevated insulin concentration.

Another mechanism that have been considered is the influence of thyroid hormones on the enzymes that are responsible for ghrelin degradation. It was demonstrated in rats that in hyperthyroidism the activity of butyrylcholinesterase is increased, whereas hypothyroidism decreases its activity (DeVriese *et al.* 2005; Legrand *et al.* 1983). The activity of the enzyme is directly connected with ghrelin concentration and might also explain the changes in ghrelin levels in subjects with thyroid disorders.

The influence of exogenous ghrelin on the concentration of thyroid hormones has also been examined and the increase of fT4 and the decrease of TSH after its administration were demonstrated (Kluge *et al.* 2010).

One study demonstrated the negative correlation between the concentration of ghrelin and the activity of anti-thyroid peroxidase and anti-thyroglobulin antibodies (Altinova *et al.* 2006).

The expression of ghrelin in the cells of the thyroid and its influence on thyroid cells were also examined. Kanamoto *et al.* studies (2001) revealed that ghrelin is produced by thyroid C-cells. These cells are known to secrete different peptides, thus it was assumed that they also secrete ghrelin. The expression of ghrelin was demonstrated in the cells of both normal thyroid and medullary cancer. The level of des-n-octanoyl ghrelin was found to be significantly higher in cancerous tissues.

Morpurgo *et al.* (2005), however, did not find any differences in ghrelin concentrations in subjects with medullary cancer before thyroidectomy, subjects after thyroidectomy, in patients with nodular goiter and in healthy subjects. However, the significant increase of ghrelin concentration in pentagastrin-stimulated calcitonin test was demonstrated and was the most significant in subjects with pathological test result. It might be the confirmation that ghrelin is secreted by thyroid C-cells.

The data considering ghrelin expression in thyroid follicular cells are contradictory. Zhang *et al.* (2006) demonstrated the presence of ghrelin in thyroid cancers, whereas in benign lesions and in normal thyroid the ghrelin was not found. The later study by Karaoglu *et al.* (2009) assessed the expression of ghrelin in normal thyroid, in thyroid papillary cancer and Hashimoto thyroiditis and demonstrated that ghrelin expression was undetectable in cancerous tissue and high in both healthy and inflammatory gland.

The presence of GHS-R was found in normal and cancerous follicular cell of the thyroid. Presumably, the stimulation of the receptor results in the inhibition of the proliferation of cancerous cell lines derived from thyroid follicular cell (Korbonits *et al.* 2001).

GHRELIN AND REPRODUCTIVE SYSTEM

Ghrelin plays an important role in both maintaining the energy balance and regulating the reproductive functions. The role of ghrelin as a factor regulating energy homeostasis has been well established. It might be thus assumed that ghrelin influences the reproductive system indirectly, since a well-balanced energetic state is vital for proper functions of the hypothalamic-pituitary-gonadal axis. Currently it is known that ghrelin also influences this axis directly. The expression of ghrelin and its receptor has been demonstrated in many structures of the reproductive system. Tena-Sempere *et al.* (2002) demonstrated the presence of ghrelin and GHS-R in rat testis. The expression of ghrelin was detected in Leydig cells and ghrelin receptor is most probably expressed in Leydig and Sertoli cells. The expression of ghrelin was found to be the highest in the testes of adult rats compared with young animals. Similar results were shown in studies on male genitalia (Gaytan *et al.* 2004) Other studies demonstrated the expression of ghrelin in the ovaries. In the study examining the rat ovary, ghrelin expression was found in the corpora lutea and lower in the cells of interstitial gland (Caminos *et al.* 2003). Moreover, it was shown that the level of ghrelin expression varied depending on the phase of the cycle, suggesting that the expression of ovary ghrelin is controlled by gonadotropins and/or ovary steroids. Another study demonstrated the presence of ghrelin and GHS-R 1a in human ovaries (Gaytan *et al.* 2003). The expression of ghrelin was studied with the use of immunohistochemical reaction. A strong reaction was demonstrated in the hilus interstitial cells of ovary. Moreover, immunohistochemical reaction was detected in granulosa-luteal cells and in mature corpora lutea. GHS-R 1a was found to be present in the oocyte, in granulose cells and thecal cells of the follicles, in the cells of forming, mature, old and

regressed corpora lutea and in interstitial cells of both the hiatus and hilus of the ovary.

In one of our study (Komarowska *et al.* 2006) we examined the expression of ghrelin in polycystic ovaries. We demonstrated that ghrelin is expressed in both polycystic and normal ovaries. In polycystic ovaries we detected stronger nuclear immunoreactivity compared with healthy ovaries. Moreover, we demonstrated that the cells with nuclear immunoreactivity present with intense proliferative activity. However, the significance of our findings is currently hard to be explained.

It seems that ghrelin is involved in the proper embryo implantation. A strong ghrelin expression was demonstrated in the forming placenta in the first trimester of the pregnancy (Tanaka *et al.* 2003). In addition, ghrelin levels were found to be decreased in the third trimester suggesting its relaxant influence on the uterus (Hehir *et al.* 2008).

The fact that ghrelin and its receptor are so widely distributed in the structures of the reproductive system confirms its significant role in the regulation of reproductive functions. More studies have been confirming that hypothesis.

In most studies conducted in animals and humans it was demonstrated that ghrelin inhibits the secretion of LH and FSH (Furuta *et al.* 2001). The effect might be exerted directly through GnRH-LH and GnRH-FSH axis or indirectly through CRH and kisspeptin. In vitro studies, however, demonstrated that ghrelin stimulates the secretion of gonadotropins depending on its dose and the phase of the cycle (Fernandez-Fernandez *et al.* 2006). Moreover, Martini *et al.* (2006) demonstrated that both acylated and non-acylated ghrelin can regulate gonadotropins secretion. In human studies ghrelin was found to inhibit the pulsatile LH secretion (Kluge *et al.* 2012; Lanfranco *et al.* 2008)

Considering that ghrelin is the signal informing about energy insufficiency, it might be assumed that the suppression of gonadotropins secretion observed in underweight subjects results at least partially from ghrelin actions. It might explain the way in which ghrelin can disturb the menstrual cycle. It's been also assumed that high concentration of ghrelin can result in puberty delay, whereas the decrease of ghrelin level might trigger the onset of puberty (Forbes *et al.* 2009; Soriano-Guillen *et al.* 2004)

The influence of ghrelin on steroidogenesis has also been studied. It was shown that ghrelin is involved in steroidogenesis by inhibiting the expression of P450scc, 3β HSD and 17β hydroksysteroid III dehydrogenase. It was demonstrated that ghrelin expression depends on the stimulatory effect of LH (Barreiro *et al.* 2002). Ghrelin and LH are antagonists in the regulation of steroidogenesis. It's been assumed that ghrelin in the cell nucleus is the local regulator which controls the influence of LH on steroidogenesis. Studies have analyzed the correlation between the concentration of androgens and ghrelin in both men and women and significances have been found in most of the studies (Pagotto *et al.* 2002; Pagotto *et al.* 2003). The studies examined both basal concentrations of ghrelin and the role of androgen and oral contraceptives in altering ghrelin concentrations. It seems that basal ghrelin levels are higher in women compared with men.

The studies examining the etiopathogenesis of PCOS point out the role of factors regulating steroidogenesis, pituitary secretory function, insulin actions as well as the factors influencing energy homeostasis. Since the correlations between these elements and ghrelin have been found, there have been studies examining them in women with PCOS. The negative correlation between BMI and ghrelin as well as between ghrelin and insulin was the common finding (Schőfl *et al.* 2002; Tschőp *et al.* 2000; Wasko *et al.* 2004). Such results are not surprising since similar correlations were demonstrated in other disorders and in healthy population. However, the comparison of ghrelin concentrations in subjects with PCOS and healthy subjects demonstrated different results. Most commonly, lower levels of ghrelin were found in women with PCOS compared with healthy women with similar BMI (Schőfl *et al.* 2002; Tschőp *et al.* 2000). In other studies however, no significant differences (Orio *et al.* 2003) in ghrelin levels were shown or higher (Wasko *et al.* 2004) concentrations of ghrelin in subjects with PCOS were found.

Similar discrepancies were found when examining the correlations between ghrelin and androgenes. It was demonstrated that the use of oral contraceptives and anti-androgens results in the increase of ghrelin concentrations (Gambineri *et al.* 2003; Sagsoz *et al.* 2009). In many, but not all, studies a negative correlation between ghrelin and androgens were found. In one study the correlation between the concentration of ghrelin and the intensity of hirsutism was demonstrated (Panidis *et al.* 2010). Discrepant results might be the consequence of different inclusion criteria used in different studies. The Rotterdam criteria leave wide discretion and women with PCOS might differ in terms of the hormonal profile and BMI.

The differences in ghrelin levels are more significant in those studies in which subjects with pronounced obesity and insulin resistance were examined (Glintborg *et al.* 2006; Pagotto *et al.* 2002; Schőfl *et al.* 2002). Presumably it is another evidence confirming the hypothesis that PCOS in lean and obese subjects is the condition based on different pathomechanisms and it's only some of the symptoms that are common.

Ghrelin is still the peptide that brings much interest to researchers. More information have been accumulated on ghrelin's actions and the regulatory mechanisms that it's involved in. However, its actions are much more complex than it's been initially suspected, mostly because there are more than one active form of ghrelin and GHS-R 1a is not the only active receptor. The explanation of the role of ghrelin in the regulation of different functions in the body is still what we need to wait for.

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