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Ghrelin - characterization, regulation of synthesis, release and possible role in reproduction

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ABSTRACT

Ghrelin is a peptide, composed of 28-amino acid residues, with a characteristic modification by n-octanoid acid, covalently linked to the serine-3. Considering a genomic position, the gene of ghrelin in humans is located on chromosome 3p25-26. Major source of ghrelin seems to be the stomach, but other tissues and organs such as: the small intestine, pancreas, pituitary gland, arcuate nucleus of the hypothalamus, kidney, placenta, lungs, testis, ovaries and adrenal glands, were reported to synthesize this peptide also. Body weight was proved to be superior to food intake for ghrelin regulation and an impaired hormone response to feeding status appeared in obese individuals. Based on experimental data, which show that ghrelin and its receptor are expressed in normal human ovary and testis, it has been proposed that ghrelin acting as either an endocrine and/or a paracrine signal, may play a major role in the endocrine network that integrates energy balance and reproduction.

KEY WORDS: feeding, ghrelin, cachexia, GH secretagogues (GHSs), testis, ovaries

INTRODUCTION

Originally, the presence of receptors for pulsatile growth hormone release in pituitary and hypothalamus of pigs and humans suggested that unidentified

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molecule acting through this neuroendocrine pathway does exist (Howard et al., 1996). In fact, ghrelin, which belongs to a group of synthetic agents, i.e. GH secretagogues (GHSs), turned out to be this missing link. It was discovered in rat stomach as a new endogenous ligand for GHS-R by Kojima and co-workers (1999). They examined several tissue extracts in a stable cell line expressing rat GHS-R (GHS receptor), and, on the base of intracellular Ca^{2+} levels, they found an especially strong receptor stimulation by stomach extracts. Further research, including purification and isolation methods, revealed multiple variants of the peptide, another type known as des-Gln14-ghrelin in rat stomach, as well as additional posttranslational products of human ghrelin (Hosoda et al., 2003). Although for the first time isolated from the stomach, it is now broadly accepted that this molecule and its receptors are widely expressed in different tissues/organs of many vertebrates, like mammals, birds, amphibia and fish. Such an ubiquitous presence is most probably related to diverse indispensable physiological functions of ghrelin.

THE STRUCTURE OF GHRELIN

Ghrelin is a peptide, composed of 28-amino acid residues, with a characteristic modification by n-octanoid acid, covalently linked to the serine-3 (Kojima et al., 1999). Such a structural feature, unique among peptide hormones, occurs regardless of the origin of ghrelin, i.e. the species, apart from a few differences like in rainbow trout and bellfrog (Kaiya et al., 2003). According to the acylation status at Ser3 position, ghrelin could be classified as a nonacylated, octanoylated, decanoylated or possibly decenoylated form (Hosoda et al., 2003). In some of these, as well as in bovine, ovine, rat, feline, bird and fish ghrelins only a slight divergence in the standard 28-amino acid length may be observed (Kojima et al., 2005). The difference between rat and human ghrelins is restricted to as little as two amino acid residues, what confirms a functional homogeneity in mammals (Kojima et al., 1999), (Figure 1). Furthermore, along with the typical acyl-modification, a conservation of amino acid sequences, especially in the NH_2 termini of mammalian ghrelins, reflects an essential role of this region for hormonal activity (Kojima et al., 2005). Although the main active variant of ghrelin seems to be the octanoylated one, it was demonstrated that the other transcriptional or posttranslational forms could have a similar potency toward GH stimulation, e.g., synthetic decanoylated ghrelin or des-Gln14-ghrelin with the Gln14 deletion (Hosoda et al., 2003). As opposed to these peptides, the nonacylated form is present at relatively high concentrations in the stomach and the blood, but it does not show the GH-releasing activity (Hosoda et al., 2000). Instead, it could be a precursor,

further modified by acylation, or the product of deacylation, and probably has some additional functions (Kojima et al., 2005). This form of ghrelin was also proved to be an antagonist of the octanoylated peptide with regard to modulation of glucose levels and insulin secretion, but at the same time both factors share other nonendocrine modes of action, e.g., cardiovascular effects (Broglio et al., 2004). As far as the amino acid sequence is concerned, ghrelin has the greatest homology with two other gastric peptides: motilin-related peptide (MTLRP) and motilin, what might suggest a common evolutionary origin of these hormones (Asakawa et al., 2001).

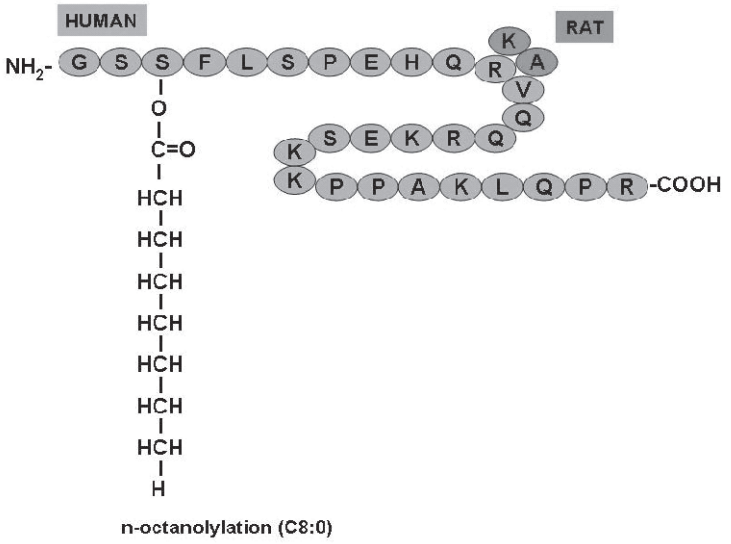

Figure 1. Structure of ghrelin in human and rat shows high level of homology. Modification at Ser3 position by n-octanoyl group, as well as the difference in amino acid sequence are depicted; according to 8, modified

Considering a genomic position, the gene of ghrelin in humans is located on chromosome 3p25-26 (Kojima et al., 2005). The latest research results show that on the level of a DNA structure, human ghrelin includes six rather than five exons, as it was primarily thought. Apart from that, suggestions regarding an existence of conserved regions in mammalian ghrelin gene were confirmed and even extended (Kanamoto et al., 2004; Seim et al., 2007). At present, it is possible to identify a broad range of gene-derived splice variants, and the number of them also significantly exceeds the one, which was expected from previous studies (Kanamoto et al., 2004; Seim et al., 2007). Consequently, obestatin is another hormone encoded by the same gene, but it has opposing effects on weight modulation (Zhang et al., 2005). It has been reported that C-ghrelin, like obestatin could be transcribed irrespective of full-

length preproghrelin (Seim et al., 2007). Some of these alternative transcripts are more prominent in particular type of cells, e.g., TT cells express Transcript-A at higher levels than Transcript-B (Kanamoto et al., 2004). Similarly, a ghrelin gene-derived transcript (GGDT) corresponding with obestatin is developmentally-regulated and specifically expressed in mouse testis (Seim et al., 2007). Interestingly, some relationships between polymorphic variants of the ghrelin gene and a still growing number of disorders, involving metabolic syndrome, obesity, anorexia nervosa, binge eating disorder, non-Hodgkin lymphoma and certain symptoms of diabetes mellitus type 2, have been observed (Monteleone et al., 2003; Steinle et al., 2005). Similarly, many animal traits, like growth, appetite, body weight, may be associated with the ghrelin gene mutations (Li et al., 2006).

GHRELIN EXPRESSION, PLASMA GHRELIN LEVEL AND POTENTIAL IMPLICATIONS

Ghrelin is produced by X/A-like cells in the oxyntic mucosa of the digestive tract. They are located nearby capillaries running through the lamina propria, act in an endocrine manner and, in such a way, enable ghrelin distribution throughout different hormone-responsive tissues (Date et al., 2000), (Figure 2).

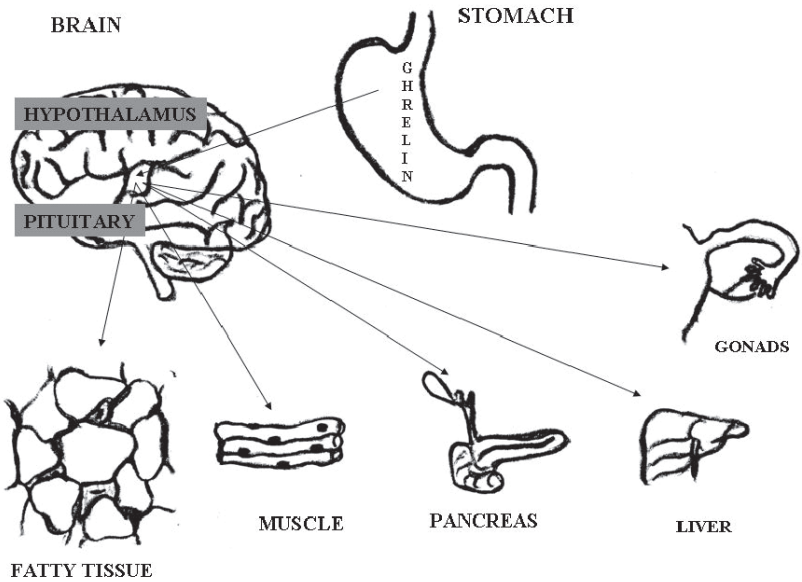

Figure 2. Major source of the plasma ghrelin is secretion from oxyntic mucosa of the stomach. Circulating ghrelin affects functions of various hormone-responsive tissues and organs, influencing among the others: GH release, energy balance, gastrointestinal and reproductive activity

In the gastric mucosa in addition to closed-type endocrine cells, open-type ones also exist, releasing ghrelin into the stomach lumen, what is thought to determine gastrointestinal functions of the hormone, for example mucosal cytoprotection, gastric motility and acid secretion (Date et al., 2000; Sakata et al., 2002b; Konturek et al., 2004). Despite the fact that the major source of ghrelin seems to be the stomach, other tissues and organs such as: the small intestine, pancreas, pituitary gland, arcuate nucleus of the hypothalamus, kidney, placenta, lungs, testes, ovaries and adrenal glands, were reported to synthesize this peptide (Kojima et al., 1999; Tena-Sempere et al., 2002b; Caminos et al., 2003). Nevertheless, there is some evidence in favour of the splanchnic bed as a substantial contributor to the circulating ghrelin concentration (Møller et al., 2003).

Although such a result appears contradictory to some findings coming from gastrectomy and fundectomy studies (Matyjek et al., 2004), at present it is difficult to fully exclude the hypothesis that a considerable decrease in ghrelin levels following surgical resection of the stomach may be modulated, to some extent, by other factors, like impaired gastrointestinal functions, alterations in food intake or a specific disease condition in patients.

Moreover, ghrelin is also expressed in several types of cancer and cancer cell lines, including breast cancer tissues and cell lines, pituitary adenomas, pheochromocytomas, pancreatic and thyroid tumors, prostate and thyroid cancer cell lines (Kanamoto et al., 2001; Iwakura et al., 2002). Elevated level of ghrelin mRNA was documented in a case of glucagonoma with multiple endocrine neoplasm type I (Iwakura et al., 2002). Furthermore, in lung cancer as well as in a murine melanoma model it was shown that plasma ghrelin levels rose in cachexia, and even increased with the progression of this condition (Shimizu et al., 2003; Hanada et al., 2004). In the study by Shimizu et al. (2003) there were no significant differences in plasma ghrelin levels between tumor-bearing patients and healthy control subjects. Concurrently, increased plasma ghrelin levels were documented only in cachectic patients and in those with anorexia after chemotherapy. Such a strict dependence on nutritional status suggests that the major contributor to upregulation observed in this case would be stomach-derived ghrelin (Shimizu et al., 2003). Accordingly, elevated levels of ghrelin and its mRNA transcript in the stomach were reported in a cancer cachexia mouse model, supporting the mode of its enhanced biosynthesis (Hanada et al., 2004). On the contrary, Garcia et al. (2005) proposed that other mechanisms, like a decreased inactivation, may influence the status of ghrelin in cachexia as well. Their analysis of active ghrelin and active to total ghrelin ratio also revealed a correlation between increased ghrelin levels and cachexia, irrespective of a particular type or stage of cancer (Garcia et al., 2005). Although molecular mechanisms regulating the hormone secretion in such a case are not fully understood, it is thought that this phenomenon may

reflect a compensatory response to metabolic imbalance correlated with a state of ghrelin resistance typical of neoplastic disease (Garcia et al., 2005). Despite this resistance there are also reports suggesting a therapeutic potential of this protein *via* its anabolic and anti-inflammatory modes of action in cancer-related cachexia (Hanada et al., 2003). It seems, however, that some positive effects, including improved feed intake and body composition may be observed only at high ghrelin doses in tumor-bearing individuals (Tena-Sempere et al., 2002b; Wang et al., 2006). The exogenous hormone therapy might turn out to be especially useful for gastric and colorectal cancer cachexia, in which plasma ghrelin level does not increase significantly (Huang et al., 2007). On the other hand, this hormone could stimulate proliferation, motility and invasiveness of certain ghrelin-responsive cancer cell lines and malignancies, like pancreatic adenocarcinomas (Duxbury et al., 2003).

An analogical situation occurs in other disorders associated with cachexia, involving chronic heart failure, chronic obstructive pulmonary disease, which are often characterized by elevated circulating ghrelin concentrations. Concurrently, supplementation of ghrelin in such a case may contribute to improvement in body composition, muscle wasting, functional capacity and sympathetic augmentation (Nagaya et al., 2006). Although these elevated hormone concentrations seem contradictory to the need for its external administration, one possible explanation has been provided by studies in patients with anorexia nervosa. Whereas plasma levels of intact ghrelin in these patients in fact vary from lower than to similar to levels in healthy individuals, there is also a visible accumulation of degraded protein variants, which would mask eventual results (Hotta et al., 2004). Additionally, an increase in fasting plasma ghrelin levels may be indicative of malnutrition states, impaired metabolism or inflammatory processes in many other pathological conditions, such as: liver cirrhosis, renal failure, celiac disease (Takahashi et al., 2006).

On the contrary, ghrelin concentration in serum decreases in a reversible manner after oesophageal substitution, as well as during acute pancreatitis and hyperthyroidism (Doki et al., 2006). Obesity is another condition associated with a reduced plasma ghrelin content in comparison with the same parameter in normally-weighted or lean individuals (Jeusette et al., 2005). Paradoxically, excessively elevated circulating ghrelin level may actually become the factor stimulating food intake, promoting hyperphagia and adipogenesis, decreasing locomotor activity, hence, potentially leading to weight gain and obesity (DelParigi et al., 2002). Therefore, for therapy, simultaneous administration of low-dose ghrelin and hypothalamic GHRH (growth hormone-releasing hormone) would be needed (Iwakura et al., 2007). Further interesting conclusions came from experiments in mice with neutralized ghrelin and ghrelin-deficient mice, which had diminished

weight gain despite a high-fat diet (Wortley et al., 2005). Body weight was proved to be superior to feed intake for ghrelin regulation (Jeusette et al., 2005) and an impaired hormone response to feeding status appeared in obese individuals (Ariyasu et al., 2002). In genetically obese animals, a delayed short-term secretion after feeding was shown, what may be mediated by blood glucose levels (Ariyasu et al., 2002). Additionally, other factors associated with obesity, like, for example hypertension, sometimes lead to elevated ghrelin levels, but only to the threshold BMI, above which there is a renewed decrease in hormone concentration despite the presence of hypertension (Öner-Iyidoğan et al., 2007). Similar situation occurs in pregnant women with pregnancy-induced hypertension, who have significantly higher levels of ghrelin in comparison with those of normal pregnant women (Kozakowski et al., 2004).

Besides pathological conditions mentioned earlier, many physiologic factors including age and gestational age for newborns, gender, pregnancy, lactation, body mass, body composition and fitness, may determine plasma concentration of ghrelin as well (Kozakowski et al., 2004; Itoh et al., 2005). An important contributor is also a response to extrinsic factors like: feed intake and caloric restriction, the type of feed, sleep duration and stress (Yokoyama et al., 2005; Yang et al., 2007). It seems that plasma ghrelin levels are negatively correlated with age and fat mass, but positively correlated with gestational age in newborns, except for those small for gestational age (Farquhar et al., 2003; Kozakowski et al., 2004). Fluctuations of ghrelin-mRNA and -protein levels from foetal life to the end of puberty probably reflect developmental changes related to feed intake and growth hormone activity (Liu et al., 2002), whereas, in case of intrauterine growth-restricted foetuses, small or hypoxic neonates, these levels might be particularly important for foetal adaptation to intrauterine malnutrition or an adverse environment, for further growth and development (Cortelazzi et al., 2003; Kitamura et al., 2003; Bruder et al., 2005). On the other hand, a visible decrease in ghrelin expression and concentration with age may contribute to diminished growth hormone production and consequently, to age associated-alterations in humans and animals (Liu et al., 2002; Kozakowski et al., 2004). Concurrently, ghrelin secretion resulting from feed intake appears to be differently regulated depending on the age of an individual (Miura et al., 2004). Circulating ghrelin levels were proved to be related to body mass not only at birth, but also later on in life (Kitamura et al., 2003; Jeusette et al., 2005). Apart from that, there are some reports indicating possible relationships between plasma ghrelin levels and body composition, especially fat mass and distribution, as well as between plasma ghrelin levels and fitness (Fagerberg et al., 2003; Kozakowski et al., 2004; Barazzoni et al., 2005). Circulating ghrelin concentrations appear negatively correlated with body fat (Fagerberg et al., 2003; Kozakowski et al., 2004), but, at the same time, elevated

ghrelin levels may promote adipogenesis and adiposity (Thompson et al., 2004). However, opposite results regarding this matter have been obtained during *in vitro* examination, in which ghrelin expression in stable 3T3-L1 cell line inhibited differentiation of preadipocytes into adipocytes and stimulated cell proliferation (Zhang et al., 2004). In accordance with these contradictory reports, it seems, that ghrelin modulates insulin-stimulated glucose uptake in selective adipocyte populations (Patel et al., 2006). Ghrelin acts in a tissue-specific manner as well, by supporting fat deposition in liver rather than in skeletal muscle (Barazzoni et al., 2005). Hence, the action of this hormone on body fat is probably more complex, mediated not only by the growth hormone secretagogue receptor (GHS-R1$_a$), but also by other types of receptors (Thompson et al., 2004; Zhang et al., 2004; Patel et al., 2006). On the other hand, in comparison of fit racehorses and unfit standard breeds, greater ghrelin concentrations were recorded in the first group (Gordon et al., 2007). In conclusion, circulating levels of ghrelin, which is a hormone involved in energy homeostasis, may be differently modulated, in order to act as a regulator of metabolic efficiency, i.e. in the control of energy intake and expenditure.

The above statement is further confirmed by the fact, that plasma ghrelin levels rise in response to fasting, whereas, in general, feed intake and oral glucose administration suppress circulating ghrelin (Yokoyama et al., 2005). Elevated circulating ghrelin and enhanced ghrelin production in the stomach were observed as a result of chronic caloric restriction in ageing mice. Such a response also supports the thesis, according to which, ghrelin functions in the regulation of negative energy balance (Yang et al., 2007). Moreover, the type of ingested nutrients influences plasma ghrelin concentration. In particular, the plasma ghrelin level is increased by high-protein content meals, while it is decreased at different time scales or to a different degree by meals rich in fat or carbohydrates (Monteleone et al., 2003).

Another factor determining ghrelin secretion and content in the blood in physiologic conditions may be sleep duration (Spiegel et al., 2004). It has been documented that sleep curtailment contributes to elevated ghrelin concentrations, and, as a consequence, to increased hunger and appetite, what eventually promotes obesity (Spiegel et al., 2004). Additionally, the stimulating effect of stress on plasma ghrelin levels, gastric ghrelin levels and expression in oxyntic mucosa has been reported (Nishizawa et al., 2006).

ROLE OF GHRELIN IN REPRODUCTION

A relationship between ghrelin expression/concentration in plasma and sex has been revealed as well, with higher levels in females (Liu et al., 2002; Kozakowski

et al., 2004). One explanation of this aspect would be a reciprocal interaction of ghrelin and sex steroids, especially androgens, that might modulate each other's level in a gender-specific manner (Kozakowski et al., 2004). In healthy men and hypogonadal men, there is a positive correlation between serum testosterone level and ghrelin level (Pagotto et al., 2003). Inhibitory effect of elevated plasma ghrelin concentration on testosterone production, which was observed in rats (Tena-Sempere et al., 2002a), probably suppresses male reproductive functions in case of energy insufficiency. Analogically, ghrelin levels in uterine fluid increase during starvation in mice, what potentially contributes to an inhibition of the embryo's development (Kawamura et al., 2003; Fernandez-Fernandez et al., 2005). In the light of these findings, a great deal of ghrelin-binding sites, that has been demonstrated in gonads, seems meaningful and indicates that ovaries and testes could play a significant role as targets of ghrelin action (Tena-Sempere et al., 2002b). Moreover, it was shown in rats that ghrelin-producing cells in the stomach differentiate earlier in females, what might suggest a possible involvement in sex development and future reproductive functions (Sakata et al., 2002a).

GHRELIN SYSTEM IN THE TESTIS

Several evidences in favour of ghrelin involvement in the regulation of testicular germ and somatic cell proliferation were presented (Barreiro et al., 2004). In the testis of sheep and rat, evident ghrelin localization has been documented in interstitial Leydig cells, as well as, albeit at lower abundance, in the germ (in sheep only) and Sertoli cells (Barreiro et al., 2002). The initial evidence for testicular expression of the ghrelin gene came from a study by Tanaka et al. (2001), who identified a testis-specific ghrelin gene derived transcript in the mouse. Thus, testicular ghrelin gene expression was demonstrated by semiquantitative RT-PCR throughout postnatal development, and ghrelin peptide was selectively detected in rat Leydig cells at advanced stages of maturation, regardless of their foetal or adult origin (Barreiro et al., 2002; Tena-Sempere et al., 2002a). Accordingly, ghrelin expression became undetectable after selective elimination of mature Leydig cells by administration of the cytotoxic compound ethylene dimethane sulphonate (EDS) (Barreiro et al., 2002; Tena-Sempere et al., 2002a). Analyses of its regulation by hormonal signals revealed that ghrelin expression in the testis is not apparently modulated by follicle-stimulating hormone (FSH), GH, thyroid hormones and glucocorticoids, but it is, at least partially, under the control of pituitary luteinizing hormone (LH) (Barreiro et al., 2002). In addition to the ligand, expression of the putative ghrelin receptor was demonstrated by a combination of semiquantitative RT-PCR and *in situ* hybridization in rat testes (Tena-Sempere et al., 2002a; Barreiro et al., 2003).

Location analyses in the rat testis by means of immunohistochemistry and *in situ* hybridization revealed a scattered pattern of distribution of GHS-R1a, with specific expression in somatic Sertoli and Leydig cells, and eventually in germ cells (Barreiro et al., 2003). Not only ghrelin expression, but also testicular sensitivity to ghrelin is likely regulated by hormonal (homologous and heterologous) signals, which is highly suggestive of a finely tuned, direct action of ghrelin in the control of testicular function. Besides expression analyses, direct biological actions of ghrelin in the rat testis have been demonstrated using a combination of *in vivo* and *in vitro* approaches (García et al., 2007). Ghrelin was proven to significantly inhibit, in a dose-dependent manner, stimulated testosterone secretion. The inhibitory effect of ghrelin upon testosterone secretion was associated with a significant decrease in human choriogonadotropin (hCG)-stimulated levels of the mRNAs encoding several key factors in the steroidogenic route, such as steroid acute regulatory protein, and the enzymes P450 side-chain cleavage, 3b-hydroxyl steroid dehydrogenase (HSD) and testis specific 17b-HSD type III. The fact that ghrelin equally decreased hCG- and cAMP-induced testosterone secretion indicates that this inhibitory action must take place in a step beyond cAMP formation (Tena-Sempere et al., 2002a). Considering that the major stimulant for testicular expression of ghrelin is pituitary LH, ghrelin might operate as a local regulator in the fine-tuning of the steroidogenic actions of LH, which might participate in the autolimitation of testicular testosterone response to gonadotropic stimulation (Barreiro et al., 2002). Alternatively, such an inhibitory effect upon testosterone secretion might be conducted by the systemic, gut-derived ghrelin, whose plasma levels are inversely correlated with body mass index (Dornonville et al., 2005). Thereby, elevated ghrelin levels (as those observed in energy insufficiency) might contribute to the suppression of male reproductive axis in situations of negative energy balance, such as starvation. In addition to its steroidogenic effects, ghrelin might also directly regulate seminiferous tubule functions, as expression of GHS-R1a was demonstrated in the tubular compartment of the testis (Barreiro et al., 2003).

GHRELIN SYSTEM IN THE OVARY

In the ovaries, the stage of the oestrous cycle affects ghrelin mRNA content, with the highest expression in the luteal phase and the most prominent concentration of the protein in the *corpus luteum*, interstitial hilus cells and in the granulosa cells (Caminos et al., 2003). It seems, however, that these fluctuations are tissue specific, and do not exert considerable influence on circulating ghrelin levels. Ghrelin mRNA levels significantly varied depending on the phase of the cycle, with the

lowest expression levels in proestrus and maximum values in the dioestrous (day 1) phase. Such a cyclic profile of expression, with peak levels in the luteal stages, is highly suggestive of predominant expression of ghrelin in the *corpora lutea* (CL) of the current cycle. Thus, ghrelin mRNA level reached their highest value when the CL entered into its functional phase and remained lower during CL formation and regression. This contention was further substantiated by immunohistochemical analyses, which showed intense and specific ghrelin immunoreactivity in the cytoplasm of steroidogenic luteal cells (Caminos et al., 2003). In addition, non-apoptotic cells in regressing CL from previous cycles and, to a lesser extent, cells from the interstitial gland showed detectable ghrelin immunostaining (Caminos et al., 2003). The profile of ghrelin expression in the CL was roughly coincidental with its peak in functional activity and paralleled the pattern of progesterone secretion (Duggal et al., 2002; Caminos et al., 2003), which is suggestive of a potential functional role of ghrelin in the regulation of luteal development and/or function in the rat. Nevertheless, whether ghrelin is actually involved in steroidogenesis, angiogenesis, tissue remodeling, and growth of the CL remains to be established. In keeping with the functional and morphological data indicative of a predominant expression of ghrelin in the CL within the cyclic ovary, blockade of the preovulatory surge of gonadotropins and subsequent ovulation by means of administration of a potent gonado-tropinreleasing hormone (GnRH) antagonist significantly disturbed the cyclic profile of ovarian ghrelin mRNA expression (Nekola et al., 1985; Caminos et al., 2003). Indeed, ghrelin mRNA levels in ovaries from rats treated with a single dose of GnRH antagonist persistently remained at values similar to those in the proestrous stage and were significantly lower than those in paired dioestrous d 1 and dioestrous d 2 cyclic ovaries (Caminos et al., 2003). It is likely that prevention of ovulation which, in turn, blocks formation of the new CL, accounted for the decrease in ghrelin mRNA expression in ovaries from GnRH antagonist-treated rats (Nekola et al., 1985; Caminos et al., 2003). Additionally, the reduction of circulating LH levels after GnRH antagonist treatment may cause the decrease in ovarian ghrelin mRNA levels.

GHRELIN IN PREGNANCY AND LACTATION

Pregnancy and lactation are characterized by changes in plasma gonadal steroids, growth hormone (GH) and insulin-like growth factor (IGF-1) concentrations. These conditions promote a mobilization of the animal's adipose tissue stores, stimulate the gluconeogenetic process and reduce the assimilation of glucose by non-mammary tissues (Noblet et al., 1990). During pregnancy, several neuroendocrine changes and energy metabolism alterations occur. In this hypermetabolic state, a great increase

in maternal body fat and weight and a positive energy balance are well recognized, primarily to prevent the depletion of maternal energy stores (Richard and Trayhurn, 1985). Ghrelin expression has been shown in human and rodent placenta and has been reported to inhibit the development of mouse pre-implantation embryos *in vitro* (Gualillo et al., 2001; Kawamura et al., 2003). Shibata et al. (2004) demonstrated that plasma ghrelin concentrations markedly decrease during pregnancy and lactation in rats and Fuglsang et al. (2005) observed that serum ghrelin levels peak around mid-gestation in pregnant women. In rats ovarian ghrelin mRNA expression is highest during early pregnancy and decreases later on (Caminos et al., 2003). As reported by Shibata et al. (2004) in rats, the maternal concentration of ghrelin in plasma markedly decreases from mid- to late gestation; this reduction may be an important event during pregnancy. As demonstrated by Fernandez-Fernandez et al. (2005) in rats, daily administration of ghrelin during the first-half of gestation does not alter the percentage of successful pregnancies at term but significantly decreases the number of pups per litter. These facts support the hypothesis that ghrelin may operate as a signal for energy insufficiency during early stages of gestation, acting as an inhibitory factor to avoid excessive metabolic drain. The decreased plasma ghrelin concentration during the later phase of pregnancy may likely represent a physiological adaptation to a condition of positive energy balance. Energy demand during lactation is very high and is met primarily by increased feed intake, although there is some mobilization of reserves, especially of lipids from adipose tissues. Itoh et al. (2005) in dairy cattle showed that plasma ghrelin concentrations are significantly higher in early lactating cows than in pregnant heifers or non-lactating cows. In addition, peak values of ghrelin-induced GH secretion have been demonstrated to be significantly higher in early lactating than in non-lactating cows (Itoh et al., 2005). In other experiments on pregnant and lactating rats (Shibata et al., 2004) and sows (Govoni et al., 2007) authors did not find any variation in active ghrelin levels during lactation.

CONCLUSIONS AND FUTURE PERSPECTIVES

Although our understanding of potential roles for ghrelin in physiological, as well as pathological conditions has evolved rapidly since the discovery of this hormone in the last decade, there is still a need for a more precise description of its action, especially using *in vivo* models. Recently, not only the functional significance of ghrelin in reproduction has been broadly discussed, but also there has appeared some evidence in favour of the involvement of this hormone in the pathogenesis of several diseases. Therefore, it seems that ghrelin could be a potential target for diagnosis and future therapy of many disorders and/or for fertility

control. At the moment, ghrelin may be used as a reliable provocative test in the diagnostics of GH deficiency. Besides, interconnection of ghrelin levels/mutational variants with certain animal traits, such as growth, appetite and body weight, will probably enable advances in the field of new breeding strategies. However, accurate knowledge on the spectrum of biological activities and physiological interactions of ghrelin is a crucial prerequisite for the evaluation of potential therapeutic perspectives and long-term benefits of its application.

This work presents selected aspects of ghrelin's structure and role in physiological and pathological conditions, focusing on its functions in reproduction. As regards the integrated regulation of reproduction and energy balance, ghrelin, in cooperation with other neuroendocrine modulators, like leptin or kisspeptin, acts at different levels of the gonadotropic axis. As a result, ghrelin may exert its major inhibitory effects during states of negative energy balance. This interdependence between reproduction and metabolic processes, which is influenced by ghrelin, could be further substantiated by the fact that changes in plasma levels of this hormone were documented in pathological conditions related to abnormal production of sexual steroid hormones, e.g., polycystic ovary syndrome or hypogonadism. Altogether, a state of dynamic equilibrium between ghrelin, its agonist and antagonists operates to functionally link the brain, adipose tissue and the reproductive system, in order to control proper energy expenditure, which is indispensable for normal reproduction.

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