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Physiological Functions and Pathology of Ghrelin

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Abstract: Ghrelin is a peptide hormone made up of 28 amino acid residues; the N-terminal serine 3 residues is modified by octanoic acid, a medium-chain fatty acid. Ghrelin is mainly secreted by the stomach and has various effects, including growth hormone release, hyperphagia, lipid accumulation, suppression of insulin secretion, and hypotensive effects. Most of these physiological effects are indispensable functions for the maintenance of homeostasis and contribute to the onset and promotion of metabolic syndrome. Accordingly, it is important to combine etiological and pathological understanding based on the biochemistry and physiology of ghrelin, which has a characteristic structure. In this manuscript, after presenting biochemical information on ghrelin, we provide an outline of its physiological function.

Keywords: Ghrelin, Ghrelin-*O*-Acyltransferase, Appetite

1. Introduction

G-protein coupled receptors (GPCRs), which are an important target of innovative drug development, are widely involved in biological phenomena such as intercellular signal transmission and cell proliferation and differentiation, and GPCRs also act as receptors for various ligands, ranging from ions to proteins. GPCRs are seven transmembrane receptors and comprise a small proportion of the entire human genome, forming the fourth largest protein family. Of the GPCRs, those with unknown ligands are known as orphan GPCRs. There are said to be approximately 300 types of orphan GPCRs, and their intrinsic ligands have facilitated the elucidation of novel biological information systems and paved the way for innovative drug development. Therefore, they have been proactively investigated mainly during the 1990s.

Meanwhile, in 1976, a report was written concerning weak growth hormone (GH) secretory activity that was mediated by a receptor and not by growth hormone releasing hormone (GHRH) (1). The GHRH signal is mediated by cyclic AMP, while the second messenger for this receptor is calcium (Fig. 1). Thereafter, the primary structure of this receptor was revealed through expression cloning in 1996, and it came to be known as growth hormone secretagogue receptor (GHS-R) (2). This receptor was a typical GPCR; therefore, a search for the intrinsic ligand of GHSR, whose existence was called into question, was performed on a global level. Most researchers focused their search on the brain because GHS-R is widely distributed in the central nervous system (2-4), but their endeavors were not fruitful. However, advances made during the investigation of the stomach of rats and humans by Kojima and Kangawa *et al*. (1999) led to the identification of ghrelin (GHRL), the intrinsic peptide that activates GHS-R (5).

In this review, we will review the biochemical and physiological knowledge of ghrelin.

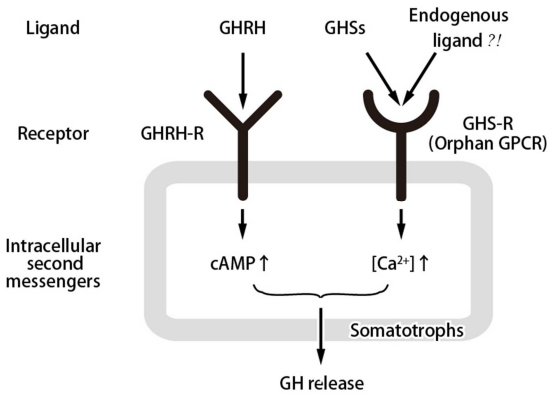

Figure 1. *Second messenger systems of GHRH and GHSs (based on Kojima et al. 2005 [6]).*

2. Biochemistry of Ghrelin and Related Substances

2.1. Ghrelin

The human ghrelin gene is present on the third chromosome at 3p25-26 (7). It is made up of six exons; ghrelin, which is made up of 28 amino acids (Fig. 2), is coded by the second and third exon (8, 9). In the ghrelin molecule, N-terminal serine 3 residues is characteristically bound via esterification to octanoic acid, a fatty acid, at carbon number 8 (Fig. 3) (5). However, bioactive peptides other than ghrelin that undergo this type of fatty acid modification have not been found in vertebrate. The amino acid sequence of ghrelin precursors has been extremely well preserved in mammals, and ghrelin has also been identified in various other species, including birds, fish, and amphibians; in all species, a fatty acid is appended to the serine 3 or threonine 3 residue (6, 9-18). The molecular weight of human ghrelin is 3370.9 kDa.

Ghrelin molecules without appended fatty acids are known as desacyl ghrelin (hereafter, "ghrelin" will refer to ghrelin that has been modified by octanoic acid). Although there are reports stating that desacyl ghrelin has an appetite-suppressing effect on the central nervous system (19), this mechanism may not be mediated by GPCR because desacyl ghrelin does not bind to GHS-R. We believe that further studies are required to clarify this issue.

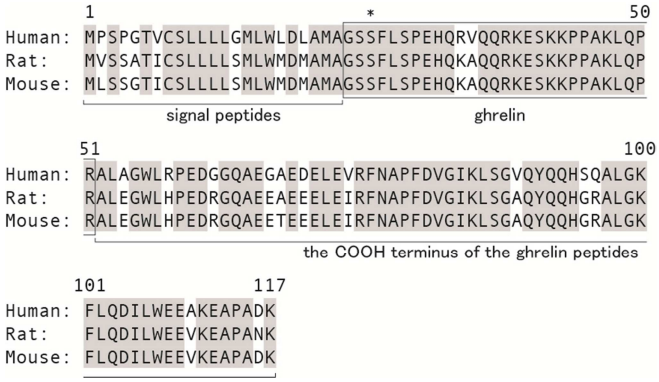

Figure 2. *Amino acid sequences of ghrelin (based on Kojima et al. 2005 [6])*

2.2. Ghrelin-O-Acyltransferase

Because fatty acid modification is essential during the expression of ghrelin activity, a proactive search was made for enzymes that appended fatty acids, and in 2008, ghrelin-*O*-acyltransferase (GOAT) was identified (20). The discovery of this enzyme triggered the discovery of an acyltransferase known as porcupine. Porcupine is an enzyme that performs fatty acid modification of Wnt, which is related to embryogenesis and cancer. When this discovery was made, a protein from a database with structural characteristics of the membrane-bound *O*-acyltransferase family was analyzed. Yang *et al*. restricted the search to 16 candidate molecules; of these, GOAT was discovered to be the enzyme that bound *n*-octanoic acid on ghrelin. Localization of this enzyme was extremely consistent with the cells that secrete ghrelin. In addition, the optimal temperature for this enzyme reaction was reported to be 37–50 °C, with an optimal pH value of 7–8 (21).

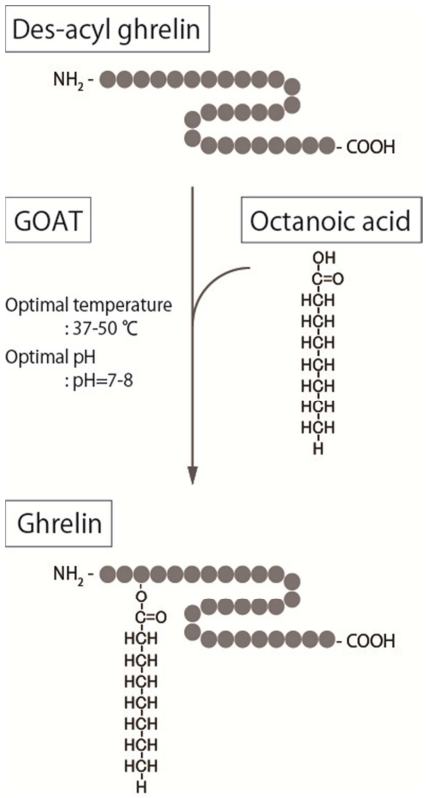

Figure 3. *Activation process of ghrelin (based on Kojima et al. 2005 [6]).*

2.3. Ghrelin Receptor

The human ghrelin receptor gene is present at the 3q26-27 on the third chromosome (7), the same chromosome with ghrelin. The human ghrelin receptor gene is made up of two exons; the first to fifth transmembrane domains are present in the first exon, and the sixth to seventh transmembrane domains are present in the second exon. Through alternative splicing, two mRNA molecules, GHS-R1a and GHS-R1b, can be produced from the ghrelin receptor gene (2). Of these, GHS-R1a functions as a GPCR with seven transmembrane domains and ghrelin receptor. On the other hand, the inactive variant of GHS-R, GHS-R1b, appears to play a critical role in modulating the activity of GHS-R1a by forming heterodimeric complexes which attenuates trafficking of the active variant to the cell surface (22). The ghrelin receptor has 52% homology with the amino acid sequence of the motilin receptor, a peptide that promotes gastrointestinal motility (23-25). The ghrelin receptor is coupled to a trimeric Gq protein and promotes release of Ca^{2+} from endoplasmic reticulum through production of inositol-3-phosphate and phospholipase activation, leading to ghrelin signal transmission. Ghrelin receptors are expressed in many tissues throughout the body, and as mentioned later, ghrelin control various physiological functions.

2.4. Obestatin

Obestatin is a peptide made up of 23 amino acid residues that was isolated from the stomach of rats (26). Reports indicate that despite being produced by cleavage from a ghrelin precursor, it demonstrates physiological effects opposite to those of ghrelin, acting as an appetite suppressant. From the results of immunohistochemical staining in rats, obestatin is reported to be present in the mucosal cells of the stomach, nerve plexuses in the gastrointestinal system, and the Leydig cells in the testes. In addition, almost all obestatin-immunopositive neurons in gastrointestinal nerve plexuses are said to co-exist with choline acetyltransferase. When obestatin was initially discovered, it gained attention as a novel appetite suppressant peptide; however, reproducibility could not be confirmed in experiments performed by various groups (27-29). Holst *et al.* reported that GPR39 signaling is stimulated by zinc ions but not by obestatin (28). Moreover, Nogueiras *et al.* failed to find any effect of obestatin on GH secretion *in vivo*, and they also unable to find mRNA expression of GPR39, the putative obestatin receptor, in the hypothalamus of rats (30). For this reason, use of commercially available obestatin EIA kits should be carefully monitored.

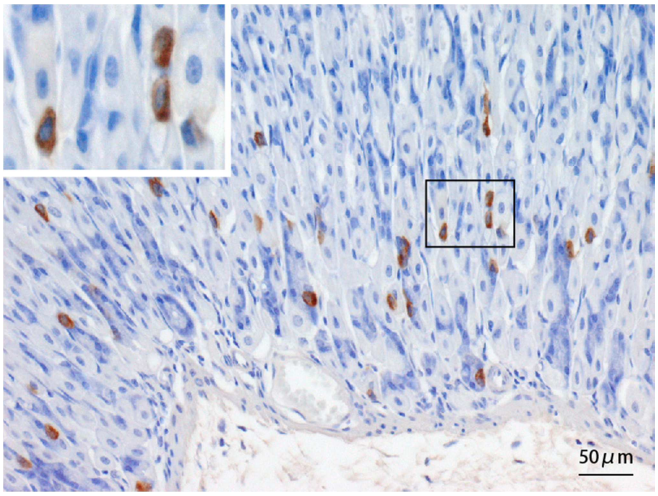

Figure 4. *Distribution of ghrelin-immunopositive cells in the mucosa of the stomach. Insert photo expansion of the black frame region.*

3. Distribution of Ghrelin and Regulation of Ghrelin Secretion

3.1. Histological Distribution of Ghrelin

The principal site of ghrelin production is the stomach (Fig. 4), particularly the fundic gland, where there are several secretory glands. This is the same for mammals and all non-mammalian species. In ruminants such as bovine and sheep that have multiple stomachs, ghrelin is produced in the glandular stomach. The gastric ghrelin cells are closed endocrine cells that are not in contact with the gastric lumen, have a roughly uniform size (diameter, 120 nm), and contain several highly electro-dense secretory granules (31). In rats, approximately 2% of the gastric mucosa is made up of endocrine cells, and of these, approximately 20–25% are ghrelin cells. These are the second most common cells after enterochromaffin-like (ECL) cells that secrete histamine.

In addition, ghrelin is also present in the duodenum, small intestine, pancreas, hypothalamus, placenta, and kidneys, although with extremely low concentrations. In the central nervous system, ghrelin-producing neurons are present in the lateral arcuate nucleus, and the nerve fibers project into other nuclei such as the median eminence and the hypothalamus (6, 32).

3.2. Plasma Concentration of Ghrelin

Ghrelin secreted by the stomach circulates in the blood stream as a hormone and acts on target tissues. Plasma ghrelin concentration in humans is 10–20 fmol/mL, and the total concentration of ghrelin and desacyl ghrelin is 100–150 fmol/mL (33-35). In rats, ghrelin concentration in the stomach is 377.3 ± 55.8 fmol/mg for *n*-octanoyl ghrelin and 1,779.8 ± 533.9 fmol/mg for total ghrelin (36). Thus, ghrelin concentration is approximately 10-25% that of desacyl ghrelin concentration.

3.3. Regulation of Ghrelin

Secretion of ghrelin is accelerated in low energy states, but the regulatory mechanism remains unknown. Even when the stomach is dilated through the intake of water, there are no changes in the plasma concentration; this demonstrates that stretching stimuli in the stomach does not change ghrelin secretion (37, 38). On the other hand, plasma ghrelin concentration is decreased by glucose administration; it increases during fasting and decreases when food is ingested (39). Therefore, plasma glucose concentration is thought to be important for the regulation of ghrelin secretion. Furthermore, in order for ghrelin to have physiological activity, fatty acid modification by GOAT is essential; therefore attention must be paid to GOAT dynamics as well as the secretion process of synthesis, storage, and release.

4. Physiological Functions and Pathology of Ghrelin

Metabolic syndrome is initiated by the accumulation of visceral fat due to excess energy intake. Energy metabolism is suppressed by intake and regulation of glycolipid metabolism, and ghrelin is involved in all of these processes. We have now mentioned the main physiological functions of ghrelin (Table 1) and will go on to discuss some of its relationships with pathological conditions.

4.1. Promotion of GH Secretion

Both *in vitro* and *in vivo*, ghrelin demonstrates powerful GH releasing effect (5, 38-45). When ghrelin was administered intravenously to healthy individuals, maximum GH release occurred after 15–20 min, and increases in the plasma GH

levels were maintained for more than 60 min (42). Furthermore, doses of ghrelin that would not stimulate GH secretion when administered alone caused very powerful GH release stimulating effects when administered with GHRH (41, 45). This shows that, in addition to being independently induced by GH secretion from GH cells, GH secretion is also stimulated by synergistic effects with GHRH. Ghrelin also stimulates the secretion of prolactin (PRL) when administered at high concentrations. Because GHS-R mRNA is expressed in the somatotrophs, mammosomatotrophs that contain both GH and PRL in the same cells, and mammotrophs of the human pituitary adenomas (46), we believe that GH and PRL are also released from mammosomatotrophs as a response to ghrelin stimulation.

Table 1. *Physiological functions of ghrelin*

Physiological functions		Species
Growth hormone secretion	↑	human, rats
Appetite regulations		
Food intake	↑	human, rats
AMPK activity	↑	rats
Autonomic nervous system		
Sympathetic nerve activity	↓	human, rats
Glucose metabolisms		
Blood glucose	↑	human
Insulin	↓	human
Lipid metabolism		
Adiposity	↑	rats
Triglyceride	↑	rats
Cardiovascular functions		
Blood pressure	↓	human, rats
Cardiac output	↑	rats
Vasodilation	↑	rats
Gastric functions		
Gastric acid secretion	↑	rats
Gastric movement	↑	rats

↑, Stimulate; ↓, decrease.

In this manner, the main function of ghrelin on GH cell function is to promote the release of GH, and we postulate that ghrelin may be involved in the GH gene expression and the development and differentiation of GH cells. In fact, it is reported that ghrelin, in addition to its GH-releasing activities, is also capable of regulating pit-1 transcription through the GH secretagogue receptor in the pituitary, suggesting the physiological roles of ghrelin on somatotroph cell differentiation and function (47).

4.2. Actions on Dietary Regulation

When ghrelin was administered centrally or peripherally in mice and rats, it causes hyperphagia and an increase in body weight (48-53). Hyperphagia caused by ghrelin is not related to the effects that promote GH secretion. Although orexigenic peptides such as neuropeptide Y (NPY) and agouti-related protein (AgRP) are only effective when they are centrally administered, ghrelin can also promote appetite through not only intracerebroventricular administration but also intravenous or intraperitoneal administration; it regulates eating solely as a peripheral hunger signal. The hypothalamus is pivotally involved in the regulation of food intake, and a large amount of information is integrated to suppress energy metabolism. Ghrelin that is administered intraventricularly activates NPY/AgRP neurons with ghrelin receptors and promotes the production and secretion of both peptides, thus causing an appetite-promoting effect (48). In addition, when ghrelin is administered intravenously, it also activates NPY/AgRP neurons and promotes food intake. The latter is dependent on the gastric vagal nerve, a cranial nerve that transmits information from the gastrointestinal system via the brainstem to the diencephalon and neocortex. Ghrelin receptors are produced in the afferent neurons of the vagus nerve and are transported to the terminal end of the afferent fibers; here, ghrelin binds to the receptor and suppresses the electrical activity of the afferent fibers of the vagus nerve (53). This information is transmitted to the medulla oblongata; thereafter, the signals switch neurons and are transported to the NPY/AgRP and GHRH neurons, producing appetite promoting effects and stimulating GH secretion (53). Leptin, which is secreted by adipocytes, suppresses NPY/AgRP neurons, and produces an appetite-suppressing effect, making it an antagonistic to ghrelin (54).

In rats, ghrelin is also secreted small amounts from the hypothalamus (32). Because chronic overproduction of ghrelin in the hypothalamus leads to temporal increase in food intake and body weight (55), ghrelin secreted from the hypothalamus also might have anabolic effects.

Plasma ghrelin concentration showed a negative correlation to body mass index (BMI). It is low in obese individuals and high in lean individuals. However, plasma concentrations in Pima Indians, who readily become obese, are low, and plasma concentrations in patients with anorexia nervosa are high. In addition, plasma ghrelin concentrations are also high in patients with severe cardiac failure or marked cachexia due to lung cancer. In Prader–Willi syndrome, which is caused by a genetic abnormality on chromosome 15, patients exhibit hyperphagia, and plasma ghrelin concentrations are also high (56). On the other hand, long-acting octreotide treatment causes a sustained decrease in ghrelin concentrations but does not affect weight, behaviour and appetite in subjects with Prader-Willi syndrome (57). The underlying mechanism for these remains unknown, but is extremely interesting.

Recently, Steculorum *et al*. reported that neonatal ghrelin programs development of hypothalamic feeding circuits (58). Direct exposure of postnatal hypothalamic neuronal explants to ghrelin blunted axonal growth and blocked the neurotrophic effect of the adipocyte-derived hormone leptin. Moreover, chronic ghrelin exposure in neonatal mice also attenuated leptin-induced STAT3 signaling in hypothalamic neurons. Thus, ghrelin regulates not only the food intake after birth, also functions to configure the neuronal network required for food intake regulation.

4.3. Effects on Glycolipid Metabolism

There are many reports on the regulation of insulin secretion by ghrelin (59-61). In a similar manner to insulin, it is also present at higher levels in the pancreatic arteries than in the pancreatic veins. Ghrelin is present in the α cells in the islets of Langerhans that produce glucagon, and the ghrelin receptor gene is expressed in both α and β cells. At physiological concentrations of ghrelin (10^{-12}–10^{-11} M), there is an increased intracellular Ca^{2+} concentration during hyperglycemia in pancreatic β cells isolated from rats, and insulin secretion is promoted. Meanwhile, during hypoglycemia, ghrelin does not change the free Ca^{2+} concentration and insulin secretion within β cells. In addition, from analysis of the pancreas of mice deficient in the ghrelin gene, it was demonstrated that glucose-induced insulin secretion from pancreatic islet cells is promoted by ghrelin deficiency (62).

While the effect of ghrelin on glucose metabolism is becoming clear, there is also cautious opinion on effect on ghrelin of glucose metabolism. Kirchner *et al.* ablated GOAT in leptin-deficient *ob/ob* mice to study whether specific ghrelin deficiency or desacyl ghrelin abundance modifies glucose tolerance on a massively obese background (63). As targeted deletion of ghrelin does not improve glucose homeostasis in their GOAT-*ob/ob* mouse model, they conclude that neither ghrelin nor the increased ratio of desacyl/acyl ghrelin is crucial for controlling glucose homeostasis in their model of massive obesity induced by leptin deficiency.

Thus, although the role of ghrelin on glucose metabolism is partly unknown, glucose tolerance improved when a GOAT-selective antagonist called GO-CoA-Tat was injected into wild-type mice that were fed a high-fat diet, and body weight gain inhibited (64). Metabolic diseases associated with obesity are a public health issue, and if these theories can be clinically applied, they would be highly significant. Treatment with GO-CoA-Tat is based on a peptide that requires repeated injection; therefore, at the present time, clinical application is difficult. However, GOAT is a potentially useful target for future drug development for obesity.

4.4. Effects on the Circulatory System

When ghrelin was administered twice daily for 3 weeks to chronic cardiac failure model rats, there were signs of an improvement in cardiac function, including decreased peripheral vascular resistance, increased cardiac output, increased left ventricular ejection fraction, suppression of the progression of left ventricular modeling, and promotion of compensatory cardiac hypertrophy in non-infarcted areas (65). In addition to these direct effects of ghrelin, there were also effects mediated by increased GH/IGF-1 caused by ghrelin. In addition, in an investigation using local administration into the anterior brachial artery in humans and GH gene-deficient pygmy rats, it became clear that ghrelin increased perfusion and decreased blood pressure in a manner independent of GH and IGF-1 (66, 67); we believe this was mediated by ghrelin receptor present in the blood vessels; therefore, ghrelin demonstrates vasodilatory effects.

When a single dose of ghrelin is administered intravenously to healthy individuals, the plasma GH concentration increases and the mean arterial pressure decreases by around 10 mmHg (68). Furthermore, cardiac index and cardiac output increase without changes in heart rate (68). The above show that ghrelin alters hemodynamics through suppression of the sympathetic nervous system. Moreover, in patients with chronic heart failure associated with cachexia, plasma GH, and ghrelin concentrations elevated (69). As plasma ghrelin concentration shows a positive correlation with BMI, plasma TNF-α, and plasma GH concentration, it is thought to play a compensatory role during a pathological condition of cachexia, where there tend to be abnormalities of energy metabolism.

4.5. Effects on the Bone

Ghrelin localized in osteoblast-like cells significantly increased their numbers and DNA synthesis in vitro. These proliferative effects of ghrelin were suppressed by [D-Lys^3]-GHRP-6, an antagonist of GHS-R1a. Furthermore, ghrelin increased the expression of osteoblast differentiation markers, ALP activity, and calcium accumulation in the matrix. Thus, ghrelin directly stimulates bone formation (70). In addition, it was reported that ghrelin also regulates bone remodeling through GHS-R in osteoblasts by modulating the cAMP response element binding protein (CREB) and runt-related transcription factor 2 (Runx2) pathways in mice (71). Moreover, chronic central administration of ghrelin increases bone mass through a mechanism that is independent of body weight, suggesting that ghrelin may have a bone anabolic effect through the central nervous system (72). On the other hand, ghrelin infusion has no acute effect on markers of bone turnover in healthy controls and post-gastrectomy subjects, which are prone to osteropenia and osteomalacia, but is inversely correlated with bone resorption (73). Thus, the effects of ghrelin on bone are not necessarily consistent results between experimental animals and humans.

5. Conclusion

By the discovery of ghrelin, the stomach has been shown to be involved in GH secretion and regulation of energy metabolism, in addition to its primary digestive function. Ghrelin was the first peptide proven to promote food intake peripherally, and clarification of its mode of action is important for elucidation of the etiology and pathology of obesity and eating disorders. It is anticipated that future research will study the mechanisms of the numerous physiological functions of ghrelin at a molecular level in order to be applied to drug discovery. Following on from this, we expect ghrelin to play an important role in innovative drug development.

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