Abstract. Ghrelin, identified as an endogenous ligand for the growth hormone secretagogue receptor, functions as a somatotrophic and orexigenic signal from the stomach. Ghrelin has a unique post-translational modification: the hydroxyl group of the third amino acid, usually a serine but in some species a threonine, is esterified by octanoic acid and is essential for ghrelin’s biological activities. The secretion of ghrelin increases under conditions of negative energy-balance, such as starvation, cachexia, and anorexia nervosa, whereas its expression decreases under conditions of positive energy-balance such as feeding, hyperglycemia, and obesity. In addition to having a powerful effect on the secretion of growth hormone, ghrelin stimulates food intake and transduces signals to hypothalamic regulatory nuclei that control energy homeostasis. Thus, it is interesting to note that the stomach may play an important role in not only digestion but also pituitary growth hormone release and central feeding regulation. We summarized recent findings on the integration of ghrelin into neuroendocrine networks that regulate food intake, energy balance, gastrointestinal function and growth.

Keywords: ghrelin, growth hormone (GH) secretagogue receptor, GH-releasing peptide, appetite regulation, stomach

I. Introduction

The pulsatile release of growth hormone (GH) from the pituitary somatotrophs is regulated by two hypothalamic peptides, growth-hormone-releasing hormone (GHRH) and somatostatin (1 – 3). GHRH is secreted by arcuate neurons into the hypothalamic portal vessels and stimulates GH release by activating GHRH receptor on pituitary somatotrophs. Somatostatin inhibits the activation of GHRH neurons through its receptor and hyperpolarizes somatotroph membranes to inhibit GH release. In addition, a third independent pathway regulating GH release has been identified from studies of GH secretagogues (GHSs) (4 – 8). GHSs are synthetic compounds that are potent stimulators of GH release, working through a new G-protein-coupled receptor (GPCR), the GHS receptor (GHS-R) (9 – 11). Because GHSs are a group of artificial compounds, it was postulated that there must exist an endogenous ligand that binds to GHS-R and carries out similar function to GHSs (12 – 14). Unexpectedly, we succeeded in the purification and identification of the endogenous ligand for the GHS-R from the stomach and named it “ghrelin” (15, 16). Ghrelin is a GH-releasing and appetite-stimulating peptide.

With the discovery of the orexigenic and adipogenic effects of ghrelin (17, 18), studies focused on defining neural circuits responsible for mediating ghrelin actions in the brain. The hypothalamus is the center for the integration of feeding and associated autonomic, neuroendocrine, and gastro-entero-pancreatic activities. Ghrelin regulates, in an antagonistic manner to leptin, synthesis and secretion of several neuropeptides in the hypothalamus that regulate feeding and energy balance. Here, we review the purification, structure, distribution, and physiological and pharmacological functions of ghrelin.
II. Discovery of ghrelin

GHSs are a family of small synthetic peptides and non-peptide molecules that stimulate the secretion of GH in several species and in humans. In 1977, Bowers and colleagues developed the first peptides of GHS derived from methionine-enkephalin, which stimulated in vitro the release of GH from pituitary cells, although their potency was rather weak (19, 20). Further development produced several more potent peptides, including GH-releasing peptide-6 (GHRP-6) and hexarelin, which had improved GH-releasing potency and were active in vitro and in vivo (8, 21). Based on the structure of GHRP-6, nonpeptidyl compounds of GHS, such as MK-0677, were developed by Merck (8).

In 1996 the Merck group made another outstanding contribution by cloning the gene for the GHS receptor, a new GPCR with seven transmembrane domains (7-TM) (9). A receptor specifically different from GHRH was strongly supported by the differences in the GH releasing activity of GHS versus GHRH as well as the pituitary intracellular signal pathways of these compounds (Fig. 1). GHRH was known to act on the GHRH receptor to increase intracellular cyclic AMP through the protein kinase A pathway (22). This indicates that the GHRH receptor is coupled to a Gs subclass of G protein. On the other hand, GHS stimulates the phospholipase C pathway, resulting in an increase in the intracellular Ca$^{2+}$ through inositol 1,4,5-triphosphate-mediated signal transduction, indicating that the ghrelin receptor is coupled to a Gq subclass of G protein (23, 24).

A cultured cell line expressing GHS-R was established and used to identify tissue extracts that could stimulate the GHS-R, as monitored by increases in intracellular Ca$^{2+}$ levels. After screening several rat tissue extracts, very strong activity was unexpectedly found in stomach extracts (15). The purified ligand from the rat stomach through four steps of chromatography procedures was a 28-amino acid peptide, and it was named ghrelin (“ghre” is the Proto-Indo-European root of the word “grow”). Ghrelin has a unique post-translational modification: the hydroxyl group of the third serine residue (Ser3) is esterified by octanoic acid and is essential for ghrelin’s biological activities (Fig. 2A). Human ghrelin is identical to rat ghrelin apart from two amino acids; however, amphibian (bullfrog) ghrelin undergo acylation at the third amino acid threonine (25). Thus, the acyl modification of hydroxyl group on the third residue represents an invariant and essential covalent change for the activation of ghrelins across multiple species (Fig. 2B). The amino acid sequences of mammalian ghrelins are well conserved; in particular, the 10 amino acids in their NH$_2$ termini are identical. In rat stomach, a second type of ghrelin peptide has been identified as des-Gln14-ghrelin (26). The ligand des-Gln14-ghrelin, a 27-amino acid peptide with an n-octanoyl group at the third threonine (25), has been identified as having similar biological activity to ghrelin (27).
octanoyl modification at Ser3, is identical to ghrelin except for deletion of one glutamine, and it is produced through alternative splicing of the rat ghrelin gene. Des-Gln14-ghrelin has the same potency of activities as that of ghrelin. In the course of purifying human ghrelin from the stomach, we also isolated several minor forms of the peptide (27). These could be classified into four groups by the type of acylation observed at Ser3: nonacylated (des-acyl ghrelin, ref. 28), octanoylated (C8:o), decanoylated (C10:o), and possibly deconoylated (C10:1). All peptides found were either 27 or 28 amino acids in length, the former lacking the COOH-terminal Arg28, and are derived from the same ghrelin precursor through two alternative pathways. Synthetic octanoylated and decanoylated ghrelins stimulate the increase of intracellular Ca\(^{2+}\) except for deletion of one glutamine, and it is produced

octanoylated and decanoylated ghrelins stimulate the increase of intracellular Ca\(^{2+}\) levels in GHS-R-expressing cells and stimulate GH release in rats to a similar degree. The nonacylated form of ghrelin, des-acyl ghrelin, also exists at significant levels in both stomach and blood (28). In blood, des-acyl ghrelin circulates in amounts far greater than acylated ghrelin. Because a fatty acid is attached to the Ser3 of ghrelin, acylated ghrelin is unstable (29). Thus des-acyl ghrelin may represent either a pre-form of acyl-modified ghrelin or the product of its deacylation. Des-acyl ghrelin does not replace radiolabeled ghrelin at the binding sites of acylated ghrelin in hypothalamus and pituitary and shows no GH-releasing and other endocrine activities in humans and rats. In contrast, it has been reported that des-acyl ghrelin shares with acylated ghrelin some nonendocrine actions, such as the modulation of cell proliferation and adipogenesis (30–32). Further study is required to determine whether des-acyl ghrelin is biologically active and binds to an as-yet-unidentified receptor.

III. Ghrelin derivatives

Based on calcium-mobilization assay in the GHS-R-expressing cell line, chemical synthesis of ghrelin derivatives revealed that bulky hydrophobic groups attached to the side chain of the third amino acid residue are essential for maximum activity of ghrelin (33, 34). When the length of the acyl modification of ghrelin was examined, the maximum response was observed at the acyl modified by the n-octanoyl group. Substantial activity was retained when ghrelin was modified by n-lauroyl or palmitoyl groups. Modification of ghrelin Ser3 by an unsaturated or a branched fatty acid, such as 3-octenoyl (C8:1) or 4-methylopentanoyl, respectively, also retained activity. Moreover, a ghrelin derivative in which the third amino acid residue was replaced with an aromatic amino acid, tryptophan, still retained weak activity. Short peptides derived from the first four residues of ghrelin, Gly-Ser-Ser(\(n\)-octanoyl)-Phe-NH\(_2\)), could activate the ghrelin receptor, but the first three alone could not, indicating that the four-residue peptide is the minimum segment necessary for receptor activation (33–35). However, the ability of the short ghrelin derivatives to activate the GHS-R expressing cells is not predictive of their capability to stimulate GH release in rats (36). The short ghrelin derivatives are devoid of biological activity in vivo.

IV. Tissue distribution of ghrelin and ghrelin receptor (GHS-R)

A. Ghrelin-producing cells

Ghrelin is predominantly produced by the stomach, whereas substantially lower amounts are derived from the bowel, pancreas, pituitary, kidney, and placenta (15, 28, 37–39). Removal of the stomach or the acid-producing part of the stomach in rats reduces circulating ghrelin by approximately eighty percent, further supporting the view that the stomach is the main source of the ghrelin peptide. These other sources of ghrelin act on ghrelin secretion in a compensatory manner after gastrectomy or might act specifically in a paracrine manner. Rat ghrelin is present from the stomach to the colon, with the greatest amount in the gastric fundus (38). Ghrelin-producing cells, which are not histamine-secreting enterochromaffin-like cells, somatostatin-secreting D cells, or serotonin-secreting enterochromaffin cells, accounted for about twenty percent of the endocrine cell population in rat and human oxytic glands (Fig. 3: A–C) (38). Rat gastric ghrelin is present in a distinct cell type, X/A-like cells, whose hormonal product and physiological functions have not previously been clarified. The X/A-like cells, now designated ghrelin-secreting cells, are not in continuity with the stomach lumen but rather are closely associated with the capillary network of the lamina propria, supporting an endocrine role. The amount of ghrelin is very low in the fetal stomach and increases in an age-dependent manner (40). The concentrations of plasma ghrelin also increase postnatally in parallel with the amount of ghrelin produced by the stomach (41). Ghrelin-immunoreactive cells are localized in the mucous membrane in the duodenum, jejunum, ileum, and colon (28, 38, 42). In the intestine, ghrelin concentration gradually decreases from the duodenum to the colon. Ghrelin cells can be classified into opened- and closed-type cells; opened-type cells are in contact with the glandular lumen, and closed-type cells do not have a luminal connection. The ghrelin cells in the stomach are closed-type cells, whereas in the duodenum, jejunum, ileum, and colon,
open-ended and closed-type of ghrelin cells are found, and the number of open-ended cells gradually increased in the direction from stomach to the lower gastrointestinal tract.

Ghrelin has been found in the hypothalamic arcuate nucleus (ARC), an important region for controlling appetite (Fig. 3D) (17, 43). In addition, a recent study has reported the presence of ghrelin in previously uncharacterized hypothalamic neurons adjacent to the third ventricle between the dorsal, ventral, paraventricular (PVN), and arcuate hypothalamic nuclei (44). These ghrelin-containing neurons send efferent fibers to neurons that contain neuropeptide Y (NPY) and agouti-related protein (AgRP) and may stimulate the release of these orexigenic peptides. These localization patterns of ghrelin suggest a role in controlling food intake. In fact, injection of ghrelin into the cerebral ventricles of rats potently stimulates food intake, and anti-ghrelin immunoglobulin G (IgG) robustly suppresses feeding (17).

The pancreas is also a ghrelin-producing organ. In the pancreatic islets, however, the cell type of ghrelin cells remains controversial, whether it be the α cells, β cells, the newly identified islet ε cells, or a unique novel islet cell type (45–49). Interestingly, the pancreatic ghrelin profile changes dramatically during fetal development; pancreatic ghrelin-expressing cells are numerous from midgestation to the early postnatal period, comprising 10% of all endocrine cells, and decrease in number after birth (50). Ghrelin mRNA expression and ghrelin concentration (mostly des-acyl ghrelin) are markedly elevated in the fetal pancreas, being several times greater than in the fetal stomach. The homeodomain protein Nkx2.2 is essential for the differentiation of islet β cells and α cells, and lack of Nkx2.2 in mice results in replacement of pancreatic endocrine cells by cells that produce ghrelin (46). The ontogenetic appearance of islet ghrelin cells precedes that of gastric ghrelin cells, which may indicate a developmental role for islet ghrelin.

B. Circulating ghrelin

To measure the plasma concentration of ghrelin, it is necessary to use EDTA and aprotinin when collecting blood samples (29). After the samples are centrifuged, the plasma fraction should be treated with 1/10 volume of 1 N hydrogen chloride. The acidified plasma should be kept in the freezer. Two major forms of ghrelin are
found in plasma: \( n \)-octanoyl-modified and des-acyl ghrelin. The normal plasma concentration of ghrelin in humans is 10 – 20 fmol/ml for mostly \( n \)-octanoyl ghrelin and 150 – 180 fmol/ml for total ghrelin, including both acyl-modified and des-acyl ghrelin. Circulating ghrelin is increased in fasting conditions and reduced after habitual feeding (51, 52), suggesting that ghrelin may be as an initiation signal for food intake or ghrelin secretion is controlled by some nutritional factors in blood.

C. Ghrelin receptor

The ghrelin receptor, or GHS-R, is a typical G-protein coupled-seven transmembrane receptor. Two distinct ghrelin receptor cDNAs have been isolated (9). The first, GHS-R type 1a, encodes a 7-TM GPCR with binding and functional properties consistent with its role as ghrelin’s receptor. Another GHS-R cDNA, type 1b, is produced by an alternative splicing mechanism (9). The GHS-R gene consists of two exons: the first exon encodes TM-1 to TM-5, and the second exon encodes TM-6 to TM-7. Type 1b is derived from only the first exon and encodes only five of the seven predicted TM domains; it is thus a COOH-terminal truncated form of the type 1a receptor and is pharmacologically inactive. The ghrelin receptor is well conserved across all vertebrate species examined, including a number of mammals, chicken, and pufferfish (13, 53, 54), suggesting that ghrelin and its receptor serve important physiological functions.

The mRNA of ghrelin receptor, GHS-R type 1a, is prominently expressed in the ARC and ventromedial nuclei and in the hippocampus (9, 17, 55). The ghrelin receptor is highly sensitive to GH; its expression is increased in GH-deficient \( dw/dw \) dwarf rats, and treatment of these rats with GH decreases ghrelin receptor expression (56). GHS-R is also detected in multiple hypothalamic nuclei and in the pituitary, as well as the dentate gyrus, CA2, and CA3 regions of the hippocampus, the substantia nigra, the ventral tegmental area, and the dorsal and median raphe nuclei. Moreover, a ghrelin-receptor transcript product is found in a mRNA sample isolated from the vagal nodose ganglion (57). Receptors in the vagus are synthesized at the cell bodies and transported to the nerve terminals through axonal transport. These results indicate that there is a close proximity between ghrelin-producing cells and vagal afferent terminals in the stomach. RT-PCR analyses demonstrated ghrelin receptor mRNA expression in many peripheral organs, including heart, lung, liver, kidney, pancreas, stomach, small and large intestines, adipose tissue, and immune cells (55, 58, 59), indicating that ghrelin has multiple functions in these tissues.

V. Physiological and pathophysiological actions of ghrelin

A. Neuroendocrine effect of ghrelin

Ghrelin is a multifaceted peptide hormone (Table 1). Because GHS is an agonist of the GHS-R, it was reasonable to expect that ghrelin possessed GH-releasing activity. In rats, large secretions of GH were observed following intravenous (i.v.), intraperitoneal, subcutaneous, and intracerebroventricular (i.c.v.) injection of ghrelin (15, 43, 60, 61), indicating that ghrelin is (directly or indirectly) a GH-releasing peptide. Intravenous ghrelin administration in healthy humans potently stimulated GH release, whereas adrenocorticotrophic hormone (ACTH), cortisol, and prolactin levels are also elevated slightly after ghrelin injection (62, 63). The coadministration of ghrelin and GHRH synergistically effect GH secretion (64).

Ghrelin stimulates GH release from primary cultured pituitary cells, which indicates that ghrelin can act directly on the pituitary (15). However, the involvement of the hypothalamus in ghrelin-mediated stimulation of GH release has been strongly suggested. Patients with organic lesions in the hypothalamic region showed insufficiency of GH release even when stimulated by ghrelin (65). Prior administration of GHRH antagonists blocks nearly all GHS-dependent GH secretion in humans (66). Moreover, when using primary pituitary cells, the ghrelin treatment increased GH release by 2 –

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Table 1. Effects of ghrelin
3 times above the basal level (15), which is lower than the level of induction seen when ghrelin is administered to rats in vivo. These facts suggest that other factors may be involved in vivo in order for the maximal level of GH release to be achieved by ghrelin administration. One possibility is transmission via the vagus nerve. Indeed, when the vagus nerves are cut in rats, the induction of GH release after ghrelin injection peripherally is dramatically decreased (57), indicating that the vagus nerve is needed for the maximal stimulatory effects of ghrelin. Peripheral administration of ghrelin induces c-Fos expression in GHRH neurons in ARC; however, this effect is canceled by chemical and surgical deafferentation of the vagus nerve. These findings imply that ghrelin elicits GH secretion from the pituitary by modulation of hypothalamic GHRH via the afferent vagus nerve system.

The effect of ghrelin on pituitary hormones is not specific to GH. Ghrelin and GHS also modulate lactotroph and corticotroph in human and animal studies (62, 67, 68). A small prolactin-releasing effect of ghrelin has been shown directly in pituitary cell cultures, so the major site of action may be either the pituitary or the hypothalamus (69). Since GHS do not stimulate ACTH release directly from pituitary cell cultures, it is probable that GHS affect the hypothalamo-pituitary-adrenal (HPA) via either one of the two major ACTH stimulators in the hypothalamus, corticotrophin-releasing hormone (CRH) and arginine-vasopressin (AVP). In a hypothalamic incubation study, GHS were found to stimulate hypothalamic AVP release while no reproducible effect was observed on CRH secretion (70). When ghrelin was used in either the same system, or in hypothalamic slices, both AVP and CRH stimulation was observed (71). Interestingly, the effect of ghrelin on ACTH secretion is even more pronounced than that elicited by GHS (62, 72). These results indicate that ghrelin stimulates the HPA axis independent of the pituitary, via the hypothalamus, involving both CRH and AVP stimulation.

**B. Ghrelin stimulates food intake**

Feeding is a basic behavior that is necessary for life. It is well accepted that appetite is controlled by the brain and that feeding behavior is regulated by complex mechanisms in the central nervous system, in particular the hypothalamus (73). GHS causes a short-lived increase in food intake when administered either systemically or i.c.v. (74, 75). The orexigenic or appetite-stimulating effect of GHS is not altered by pretreatment with a GHRH antagonist at a dose that completely blocked the feeding response to i.c.v. administered GHRH, indicating GHS-R mediates the effect on appetite. Both the peripheral and central administration of ghrelin also stimulated food intake in freely feeding rodents and in GH-deficient dwarf rats (17, 18, 43, 61). A GHS-R antagonist suppressed ghrelin-induced feeding and furthermore, the administration of ghrelin-specific antibodies suppressed starvation-induced feeding in a dose-dependent manner, suggesting that ghrelin is a powerful, endogenous orexigenic peptide. In humans, i.v. bolus injection or infusion of ghrelin induces hunger (76). Chronic i.c.v. administration of ghrelin strongly stimulates feeding in rats and increases body weight gain (17). Daily subcutaneous administration of ghrelin in mice induces a progressive increase in body weight, with a significant gain in fat mass but no change in lean body mass. This could result from a chronic decrease of fat oxidation as indicated by an increased respiratory quotient (18). Because ghrelin can induce adiposity that is sustained during ghrelin treatment, ghrelin might participate in the long-term regulation of body mass.

Unlike ghrelin, most other hypothalamic peptides, for example, NPY, AgRP, orexins, melanin-concentrating hormone (MCH), and galanin, that stimulate feeding when administered centrally are ineffective when administered into the periphery. Ghrelin is the first identified circulating hormone that promotes feeding following systemic administration.

**C. Central actions of ghrelin**

Immunohistochemical analyses indicate that ghrelin-containing neurons are found in the ARC of the hypothalamus, a region involved in appetite regulation (15, 77). In the ARC, these ghrelin-containing neurons send efferent fibers onto NPY- and AgRP-expressing neurons to stimulate the release of these orexigenic peptides and onto pro-opiomelanocortin (POMC) neurons to suppress the release of this anorexigenic peptide. The neural network of ghrelin in the PVN is more complex. In the PVN, ghrelin neurons also send efferent fibers onto NPY neurons, which in turn suppress γ-aminobutyric acid (GABA) release, resulting in the stimulation of CRH-expressing neurons, leading to ACTH and cortisol release.

As suggested by the distribution of ghrelin-containing neurons in the hypothalamus, i.c.v. administration of ghrelin induces c-Fos expression in regions of primary importance in the regulation of feeding, including ARC, PVN, and dorsomedial and ventromedial hypothalamic nuclei (17). This distribution is coincident with that of GHS-R (55). GHS-R mRNA is expressed in 94% of the neurons in the ARC that express NPY, in 8% of cells that express POMC, in 30% of those that express somatostatin, and in 20–25% of those that express...
GHRH mRNA (78). Moreover, i.c.v. administration of ghrelin leads to increases in the expression of both NPY and AGRP mRNAs (17, 43). The appetite-stimulating effects of ghrelin are blocked by an antagonist of NPY-receptor 1. I.c.v. injection of an AgRP inhibitor, anti-NPY IgG or anti-AgRP IgG, inhibits ghrelin-induced feeding. These results indicate that ghrelin exerts its feeding activity by stimulating NPY/AGRP neurons in the ARC to promote the production and secretion of NPY and AgRP peptides. The ARC is a crucial target of ghrelin to stimulate food intake and GH release, and the activation of NPY-producing and GHRH-producing neurons. A similar effect was observed when capsaicin, a specific afferent neurotoxin, was applied to vagus nerve terminals to induce sensory denervation. These results indicate that the vagus afferent nerve is the major pathway conveying peripheral signals of ghrelin for starvation and GH secretion to the brain.

D. Vagus nerve and ghrelin

Peripheral injection of ghrelin stimulates hypothalamic neurons and stimulates food intake (57, 76, 82). In general, peptides injected peripherally do not pass the blood-brain barrier. The detection of ghrelin receptors on vagal afferent neurons in the rat nodose ganglion suggests that ghrelin signals from the stomach are transmitted to the brain via the vagus nerve (57, 83). Indeed, vagotomy inhibits the ability of ghrelin to stimulate food intake and GH release, and the activation of NPY-producing and GHRH-producing neurons. A similar effect was observed when capsaicin, a specific afferent neurotoxin, was applied to vagus nerve terminals to induce sensory denervation. These results indicate that the vagus afferent nerve is the major pathway conveying peripheral signals of ghrelin for starvation and GH secretion to the brain.

E. Cardiovascular functions

The gene expression of both ghrelin and its receptor has been demonstrated in heart and aorta (58, 84). In addition, a radiolabeled ghrelin was shown to bind to heart and to peripheral vascular tissue, where the density of ghrelin receptor is up-regulated with atherosclerosis (85). On the other hand, considerable specific binding of radiolabeled peptidyl GHS, such as [{\textsuperscript{125}}I]Tyr-Ala-hexarelin, is easily detectable in rat myocardium and various cardiovascular tissues (86). This binding is inhibited by unlabeled Tyr-Ala-hexarelin, hexarelin, and other peptidyl GHS, but not by the non-peptidyl GHS MK-0677 (87). Therefore, these binding sites are unlikely to be classical GHS-R because they do not bind ghrelin.

There is already evidence that ghrelin and/or GHS mediate GH-independent cardiovascular functions, both in humans and in animals. In humans an intravenous administration of ghrelin decreases mean arterial pressure without changing the heart rate (63, 84). Ghrelin also increases the cardiac index and stroke volume indices. Rats with chronic heart failure (CHF) that were treated with ghrelin showed higher cardiac output, stroke volume, and left ventricular construction compared with placebo-treated controls (88). Furthermore, ghrelin increased the diastolic thickness of the non-infarcted posterior wall, inhibited left ventricle enlargement, and increased left ventricular fractional shortening in these CHF rats. Ghrelin, thus, improves left ventricle dysfunction and attenuates the development of left ventricle remodeling and cardiac cachexia.

It has been reported that ghrelin inhibits apoptosis of primary adult and H9c2 cardiomyocytes and endothelial cells in vitro (89). These effects are regulated through activation of extracellular signal-regualted kinase-1/2 and Akt serine kinase. Interestingly, des-acyl ghrelin is similarly active, and H9c2 cardiomyocytes exhibit no gene expression of the ghrelin receptor, indicating that another unidentified receptor may be involved.

F. Gastro-entero-pancreatic functions

Intravenous administration of ghrelin dose-dependently increases gastric acid secretion and stimulates gastric motility and emptying (43, 90). These responses to ghrelin were abolished by pretreatment with either atropine or bilateral cervical vagotomy, but not by a histamine H{sub 2}-receptor antagonist. I.c.v. injection of ghrelin also increases gastric acid secretion in a dose-dependent manner and induces c-fos expression in the nucleus of the solitary tract and the dorsomotor nucleus of the vagus nerve (91). The gastro-prokinetic activity of ghrelin is independent of its GH-releasing effect and is likely to be mediated by the vagal-cholinergic...
muscarinic pathway. Ghrelin accelerates the normal emptying process in rodents at doses compatible to those required to stimulate appetite and GH release (92). Ghrelin also accelerates the transit of the small intestine but not that of the colon. It was reported that calcitonin gene-related peptide (CGRP) [8 – 37], an antagonist of CGRP, is among the few agents with beneficial effects on the inhibition of gastric emptying after abdominal surgery (93). Ghrelin is more active than CGRP [8 – 37] and is capable of reversing the postoperative gastric ileus.

The identification of the pancreatic ghrelin-producing cells is a matter of controversy, as described in the section on ghrelin distribution. The role of ghrelin in insulin secretion is likewise under debate; ghrelin has been shown to inhibit insulin secretion in some experiments and stimulate insulin release in others (41, 45, 94, 95). These discrepancies may be due to experimental design and/or species differences.

VI. Control of ghrelin secretion and associated diseases

A. Regulation of ghrelin secretion

Circulating ghrelin levels are responsive to acute and chronic energy imbalance, increased by food deprivation and energy restriction, and decreased by food consumption and obesity. It is not clear what factors are involved in the regulation of ghrelin secretion. Blood glucose level may be critical; oral or intravenous administration of glucose decreases plasma ghrelin concentration (96, 97). Because gastric distension by water intake does not change ghrelin concentration, mechanical distention of the stomach alone clearly does not induce ghrelin release. In contrast, the ghrelin peptide contents in the stomach significantly decrease after fasting and increase after re-feeding. This inverse pattern of ghrelin levels in the stomach tissue and plasma may result from increased secretion of ghrelin from the stomach in response to fasting and subsequent decreased secretion upon resumption of feeding. Plasma ghrelin concentration showed a nocturnal increase, and this increase was blunted in obese subjects or by sleep deprivation (98, 99). Circulating ghrelin also decreases in patients with short bowel syndrome.

Obesity is characterized by a blunted GH secretion that might help to maintain the state and this decreased secretion is reversed by weight loss (100). Fasting plasma ghrelin levels in obesity are significantly lower than those in control subjects, and is negatively correlated with body mass index (BMI), percent body fat, and fasting insulin and leptin levels (101). The low levels of ghrelin might contribute to the decreased GH secretion and increased food consumption in obese subjects. Plasma ghrelin levels are also negatively correlated with plasminogen activator-1 levels that are elevated in insulin-resistant subjects (102). Gastric bypass surgery is an important treatment for morbid obesity that can produce prolonged weight reduction (103). Recent research has revealed that ghrelin may contribute to the body weight reduction that occurs following gastric bypass. In gastric bypass patients, ghrelin secretion was found to be reduced by up to 77% compared with the normal-weight control group and by up to 72% compared with the matched obese group (104). Furthermore, the normal meal-related fluctuations and diurnal rhythm of ghrelin level were absent in these patients. The mechanism for decreasing circulating ghrelin in gastric bypass patients is not known. Conversely, bypass subjects exhibited normal postprandial insulin secretion and diurnal leptin cycling. Therefore, suppression of plasma ghrelin by gastric bypass can contribute to the efficacy of this procedure as weight reduction therapy, in addition to the restriction of the stomach volume and the reduction of nutrient digestion.

Exogenous treatments with somatostatin and its analogs as well as infusion of urocortin-1, a potent anorexigenic peptide, suppress circulating ghrelin (105 – 107). However, administration of leptin does not modify ghrelin levels (108). Exogenous GH decreases stomach ghrelin mRNA expression and plasma ghrelin levels, but does not affect stomach ghrelin stores (109). These results suggest that pituitary GH exhibits a feedback regulation on stomach ghrelin production.

B. Polymorphism in the ghrelin gene and obesity

In humans, for two polymorphisms, Arg51Gln and Leu72Met, allele frequencies are similar between obese patients and controls (110, 111). However, it has been reported that obese patients with the Met72 allele became obese earlier than patients homozygous for the wild-type Leu72 allele, suggesting that the polymorphism may affect ghrelin’s activity. The Arg51Gln mutation results in a change in the COOH-terminal processing site of the ghrelin peptide within its precursor from Pro-Arg to Pro-Gln, resulting in the failure of the normal cleavage necessary to produce the 28-amino acid ghrelin. A 94-amino-acid-long proghrelin peptide may still be produced, although its biological activity has not been assessed.

C. Feeding disorders and cachexia

Plasma ghrelin levels in anorexia nervosa (AN) patients are high and return to control levels after weight gain by renutrition (37, 112, 113). AN patients often show markedly elevated GH levels, which may be due to
high circulating levels of ghrelin. Prader-will syndrome (PWS) is a complex genetic disorder characterized by mild mental retardation, hyperphagia, short stature, muscular hypotonia, and distinctive behavior features. Excessive appetite in PWS causes progressive severe obesity, but the mean plasma level of ghrelin is higher by three- to four-fold in PWS than control subjects (114). It is unclear what underlies the increased ghrelin levels in these patients. Elucidation of the precise mechanism by which ghrelin gene expression is regulated may reveal the genetic cause of hyperphagia in PWS.

Recent studies suggest that ghrelin may be a clinical marker of catabolism (115). Elevated plasma ghrelin levels are observed in cachexia associated with chronic heart failure, lung cancer, and liver cirrhosis. Indeed, in patients with prolonged and severe illnesses, GHS reverses diet-induced catabolism and improves alterations in the somatotrophic axis and protein catabolism.

VII. Clinical implication

The combined administration of GHS and GHRH appears to be the most potent stimulus of GH release in humans. It may be a convenient, safe, and reliable test for the diagnosis of GH deficiency. The lack of GH is associated with alterations in body composition, increased prevalence of cardiovascular diseases, and shortened life expectancy. Most of the patients with GH deficiency respond to GHS (116). Potential targets for ghrelin and GHS compounds include children and adults with GH deficiency. Chronic treatment of elderly with MK-0677, non-peptidyl GHS, is reported to reverse age-related changes of the GH/IGF-1 axis and to improve the quality of sleep in healthy elderly subjects (117). Furthermore, ghrelin has been shown to regulate expression, of a pituitary-specific transcription factor, Pit-1, suggesting a role in somatotroph differentiation in addition to GH secretion (118).

At present, ghrelin is only a peripheral orexigenic signal that is effective upon its intravenous injection. Ghrelin may be useful as an orexigenic agent for the treatment of eating disorders such as AN. In contrast, blocking or neutralizing the orexigenic action of ghrelin may be a reasonable approach to reversing a chronic obese state. However, appetite is regulated by numerous factors that may interact with and compensate for each other; indeed, ghrelin-null mice as well as NPY-null mice showed no obvious abnormalities in feeding behavior (119, 120).

In humans, ghrelin and GHS possess beneficial cardiovascular effects. In fact, administration of ghrelin in normal subjects and even in patients with chronic heart failure significant reduces cardiac afterload and increases cardiac output without increasing heart rate (88); and in rats with heart failure, it improves cardiac function and attenuates the development of cardiac cachexia. In vitro, ghrelin and GHS inhibit apoptosis of cardiomyocytes and endothelial cells (89). These results suggest that ghrelin has cardiovascular protective effects and regulates energy metabolism through GH-dependent and -independent mechanisms. Thus, ghrelin may be a new therapeutic agent for the treatment of severe chronic heart failure.

A recent study indicates a potent protective effect against ethanol-induced gastric lesions by central ghrelin and a partial peripheral protective effect (121). This effect of ghrelin is mediated by endogenous nitric oxide release and requires the integrity of sensory nerve fibers. Abdominal surgery inhibits gastric emptying and digestive motor activity in experimental animals and humans (92). Interestingly, the potent prokinetic activity of ghrelin has been confirmed and extended to allow for reversal of a gastric postoperative ileus in the animal model. It should be examined whether ghrelin can help prevent the ileus by abdominal surgery in humans.

The isolation of ghrelin can be considered a landmark in the GH field, allowing for new insights into understanding the regulation of the GH system, food intake, body fat composition and gastrointestinal functions. Ghrelin may prove to be an effective probe for elucidating the normal and pathological regulation of GH release and appetite in humans, and it should be explored in the clinical setting as a potential diagnostic and therapeutic tool.

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Aspects of Ghrelin


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Hosoda et al. 410


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