ROLE OF GHRELIN GENE-DERIVED PEPTIDES IN THE CONTROL OF ENERGY BALANCE AND CARDIOMETABOLIC HOMEOSTASIS

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Abstract

The human GHRL gene encodes a 117-aminoacid peptide, preproghrelin that is proteolytically processed to yield two peptides: ghrelin and obestatin. Ghrelin is secreted to the bloodstream in two major forms, acylated ghrelin (with an n-octanoylation in the third serine residue) and desacyl ghrelin (the form lacking n-octanoylation). Ghrelin acylation, promoted by ghrelin O-acyltransferase (GOAT), is essential for binding to the growth hormone (GH) secretagogue receptor (GHS-R) 1a and for the main endocrine functions of acylated ghrelin, including the stimulation of GH release, induction of food intake and stimulation of adipogenesis. Although devoid of binding to GHS-R 1a, desacyl ghrelin also displays orexigenic and adipogenic actions, whereas the role of obestatin in the regulation of energy balance remains unclear. The discovery of the widespread distribution of ghrelin and its receptor in the cardiovascular system opened a new research field in the role of ghrelin in the control of blood pressure and myocardial function. Indeed, ghrelin inhibits the apoptosis of cardiomyocytes and endothelial cells, ameliorates left ventricular function and reduces fibrosis after myocardial injury in experimental models. In humans, ghrelin improves endothelial function by increasing nitric oxide (NO) bioavailability, normalizes the altered balance between endothelin-1 and NO in patients with metabolic syndrome and exerts performance-enhancing effects on myocardial function of patients with chronic heart failure. This review focuses on advances in cardiometabolic effects of ghrelin gene-derived products in rodents and humans, and the possible role of ghrelin as a therapeutic molecule for treating cardiometabolic diseases.

Keywords: ghrelin, obestatin, obesity, adipogenesis, cardiometabolic diseases, left ventricular remodelling

Introduction

The adipose tissue acts as an active endocrine organ, secreting a large number of hormones, growth factors, enzymes, cytokines, complement factors and matrix proteins, collectively termed adipokines (1,2). The physiological and pathophysiological relevance of adipokines in the homeostasis of the cardiovascular system resides in their effects on blood pressure, fibrinolysis, angiogenesis, coagulation, vascular remodelling, insulin sensitivity and immunity, among others (1,3,4). In this respect, adipokines participate either directly or indirectly in the regulation of several processes that contribute to the development of inflammation, atherogenesis, hypertension, and insulin resistance (2).

Obesity was classified as a major modifiable risk factor for cardiovascular diseases by the American Heart Association and, by the American College of Cardiology guidelines for secondary prevention of coronary artery disease (5,6). Upper body excess adiposity is associated with increased incidence of cardiometabolic diseases.
(CMD) including atherosclerosis, hypertension, type 2 diabetes mellitus and the metabolic syndrome (7-9). Moreover, it has been recently described that percentage of body fat is positively associated with well-known circulating cardiovascular markers (10). The molecular mechanisms underlying the onset of obesity-associated CMD have not been completely disentangled. In this sense, ghrelin gene-derived products might constitute an important link between both pathologies.

Ghrelin constitutes a peptide hormone that stimulates appetite and induces a positive energy balance, leading to body weight gain (11,12). Additionally, ghrelin exerts several cardiovascular effects, including cardioprotective effects against ischemia, enhancement of vasodilation, cardiotoxic effects, and regulation of blood pressure (13). The present review focuses on advances in cardiometabolic effects of ghrelin gene-derived products in rodents and humans.

1. Discovery of the components of the ghrelin system
1.1. Growth hormone (GH) secretagogue receptor (GHS-R)
GHS-R was cloned from the pituitary and arcuate, ventromedial and infundibular hypothalamus of swine and humans in 1996 (14). GHS-R is a seven transmembrane G-protein coupled receptor that acts on the pituitary gland and hypothalamus to stimulate GH release (14). The human GHSR gene maps on chromosome 3, at 3p26.25, comprises 4 exons and encodes a 117-aminoacid polypeptide called proghrelin (Fig. 1) (25,26). This polypeptide contains a 23-aminoacid sequence signal at the N-terminal region, which is first cleaved by a putative signal peptidase resulting in proghrelin with 94 aminoacids. The prohormone convertases 1 and 3 (PC1/3), PC2 and furin are the endoproteases responsible for the cleavage of proghrelin after Arg28, generating the N-terminal mature 28-aminoacid ghrelin peptide as well as the C-terminal fragment, called obestatin (25,27). After this cleavage, the lysine residue of obestatin is cleaved by carboxypeptidase E. Two major forms of ghrelin are present in plasma and stomach: acylated ghrelin (~5% of total ghrelin with the n-octanoyl modification at Ser3) and desacyl-ghrelin (~95% without the acylation) (28). Other shorter forms of ghrelin have been recently described, like des-Gln14-ghrelin, but their role remains unknown (29).

1.2. G-protein-coupled receptor 39 (GPR39)
The G-protein-coupled receptor 39 (GPR39) was cloned together with another receptor, GPR38, from human foetal brain cDNA in 1997 (17). In 2005, Zhang and colleagues reported that GPR39 was the receptor for obestatin, a peptide encoded by the ghrelin gene (18). Nevertheless, other authors have failed to reproduce GPR39-binding ability to obestatin in vitro (18, 19). Current evidence is weighted heavily against a role for obestatin as a GPR39 agonist. The human GPR39 gene is located on chromosome 2, at 21q21-q22, and generates two transcripts by alternative splicing; GPR39-1a and 1b (20). The transcript GPR39-1a encodes a full-length receptor of 435 aminoacids, whereas the GPR39-1b encodes a protein that is identical in sequence to the full-length receptor between aminoacids 1–285, but after Leu285, the GPR39-1b transcript encodes Ser-Glu-Ser, followed by a stop codon; GPR39-1b is assumed to be non-functional (21). GPR39-1a is expressed selectively throughout the gastrointestinal tract, including the liver and pancreas as well as in the kidney and adipose tissue, whereas the truncated GPR39-1b form has a widespread expression, including the central nervous system, with the highest expression in the stomach and small intestine (20).

1.3. Ghrelin gene products (acylated ghrelin, desacyl ghrelin and obestatin)
In 1999, the endogenous ligand for GHS-R, ghrelin, was isolated from rat stomach (11). Ghrelin is a 28-aminoacid peptide hormone, synthesized by X/A-like cells in the mucosa of the gastric fundus (11, 22). Stomach and intestine constitute the two major ghrelin-secreting tissues (11, 22, 23), but ghrelin is also synthesized to a lesser extent in other tissues, including pancreas, kidney, gonads, heart or adipose tissue (24). The human GHRL gene maps on chromosome 3, at 3p26.25, comprises 4 exons and 3 introns and encodes a 117-aminoacid polypeptide called proghrelin (Fig. 1) (25,26). This polypeptide contains a 23-aminoacid sequence signal at the N-terminal region, which is first cleaved by a putative signal peptidase resulting in proghrelin with 94 aminoacids. The prohormone convertases 1 and 3 (PC1/3), PC2 and furin are the endoproteases responsible for the cleavage of proghrelin after Arg28, generating the N-terminal mature 28-aminoacid ghrelin peptide as well as the C-terminal fragment, called obestatin (25,27). After this cleavage, the lysine residue of obestatin is cleaved by carboxypeptidase E. Two major forms of ghrelin are present in plasma and stomach: acylated ghrelin (~5% of total ghrelin with the n-octanoyl modification at Ser3) and desacyl-ghrelin (~95% without the acylation) (28). Other shorter forms of ghrelin have been recently described, like des-Gln14-ghrelin, but their role remains unknown (29).

1.4. Ghrelin O-acyltransferase (GOAT)
Yang (30) and Gutiérrez (31) independently reported the enzyme that catalyzes the octanoylation of ghrelin in the endoplasmic reticulum, the ghrelin O-acyltransferase (GOAT), in 2008. GOAT is a porcupine-like enzyme that belongs to the family of membrane-bound O-acyltransferases (MBOAT), previously known as MBOAT4 (32). The expression sites of GOAT include stomach, intestine, colon and testis in rodents, and mainly in
Ghrelin plays a major role in the short- and long-term regulation of appetite and body weight, in addition to its ability to stimulate GH release (12,36-38). Circulating ghrelin levels are characterized by a preprandial rise and a postprandial fall, supporting its role in meal initiation (39). Moreover, total serum ghrelin concentrations increase steadily during long-term fasting in rodents and humans (40), and are reduced after refeeding (40). In addition, administration of exogenous ghrelin stimulates appetite and increases food intake by the stimulation of hypothalamic neuropeptide Y/agouti-related peptide neurons expressing GHS-R 1a (41). The molecular mechanisms underlying the orexigenic effects of ghrelin involve the activation of hypothalamic AMP-activated protein kinase and inactivation of the de novo fatty acid synthetic pathway, resulting in decreased malonyl-CoA levels and leading to the activation of carnitine palmitoyltransferase 1 (42). Despite its orexigenic effect, obesity, insulin resistance, type 2 diabetes or the metabolic syndrome are associated with a paradoxical decrease in circulating total ghrelin levels (11,43,44). Nevertheless, these pathologies are associated with a dramatic reduction of plasma desacyl ghrelin concentrations, the most abundant circulating isoform of the hor-

**Figure 1. Synthesis and processing of ghrelin gene-derived peptides.** Ghrelin is synthesized in the X/A-like cells of the mucosa of the gastric fundus. The human *GHRL* gene is located on chromosome 3 and comprises 4 exons and 3 introns. The *GHRL* gene encodes a polypeptide of 117 aminoacids, called preproghrelin. This precursor has a signal peptide that is cleaved by the action of peptidases resulting in the proghrelin molecule. This peptide is processed by PC 1 and 3 (PC1/3), PC2, furin and carboxypeptidase E, resulting in two peptides, obestatin and desacyl ghrelin. GOAT is the enzyme responsible of the esterification of an octanoic acid at Ser3 residue in ghrelin peptide.
mone, while plasma levels of acylated ghrelin remain unchanged or increased (45,46).

It was initially proposed that, contrary to the orexigenic effects of ghrelin, obestatin suppressed food intake and decreased body weight gain in rats, acting through the ghrelin receptor family member GPR39 (18). Obestatin levels are increased in fasted rats and, after re-feeding, plasma obestatin levels are significantly decreased but recovered quickly (40). Nevertheless, current knowledge of the secretory profile of obestatin is inadequate to make a correlation with specific feeding-related behaviours (47). In addition, obestatin degrades rapidly in serum and, unlike ghrelin, is not able to cross the blood-brain-barrier by a saturable transport system to allow the peptide to interact with its putative receptor GPR39 in the brain (48). Moreover, Nogueiras and colleagues failed to observe any effect of obestatin on food intake, body weight, body composition, energy expenditure, locomotor activity, respiratory quotient, or hypothalamic neuropeptides involved in energy balance regulation in rats (19). Therefore, the role of obestatin in the regulation of food intake remains unclear, and further investigations are required.

2.2. Adipogenic effects of ghrelin and obestatin

The adipose tissue also constitutes an important target for the adipogenic actions of ghrelin in rodents and humans (45). Ghrelin, GOAT and the receptors of ghrelin-related peptides, GHS-R 1a and GPR39, are expressed in human adipose tissue, suggesting an autocrine/paracrine effect of ghrelin in this tissue (45, 49-51). GHRL gene expression increases during adipogenesis and preproghrelin knockdown reduces insulin-mediated adipogenesis in 3T3-L1 adipocytes (52). Acylated ghrelin modulates preadipocyte proliferation and promotes their differentiation to mature adipocytes by increasing the expression of master adipogenic factors, peroxisome proliferator-activated receptor γ (PPARγ) and sterol regulatory element-binding protein (SREBP) (45, 53). In this sense, acylated and desacyl ghrelin directly stimulate the expression of several fat storage-related proteins, including acetyl-CoA carboxylase, fatty acid synthase, and lipoprotein lipase through both central mechanisms (54) and directly acting on human visceral adipocytes (45), thereby stimulating intracytoplasmic lipid accumulation. In addition, acylated ghrelin, desacyl ghrelin and obestatin attenuate isoproterenol-induced lipolysis through phosphatidyl-inositol-3 kinase (PI3K)-dependent mechanisms in murine 3T3-L1 cells as well as in isolated rat visceral adipocytes (55, 56). It has been recently discovered an antagonist of GOAT that reduces body weight in wild-type mice but not in ghrelin-deficient mice (57).

Recent in vitro studies suggest that obestatin also modulates adipocyte metabolism and adipogenesis. Obestatin expression is increased during adipocyte differentiation and obestatin treatment in 3T3-L1 adipocytes, in combination with isobutyl-methyl-xanthine and dexamethasone, regulates the expression of the transcription factors PPARγ and CCAAT-enhancer-binding proteins α, β, λ (C/EBPa, C/EBPβ, C/EBPγ), and hence promotes adipogenesis (58). Obestatin also decreases lipolysis in 3T3-L1 cells (56).

3. Role of ghrelin gene-derived peptides in the control of the cardiovascular system

Ghrelin acts on the pituitary and hypothalamus to stimulate GH release, while GH and its mediator, insulin-like growth factor (IGF)-1, are anabolic hormones that are essential for myocardial development and performance. Nevertheless, ghrelin has cardiovascular effects through GH-dependent and -independent mechanisms, as explained below. The analysis of ghrelin binding sites in the cardiovascular system by Katugampola and colleagues (59) showed a higher density of receptors in the myocardium of the right atrium than in the left ventricle (LV), whereas aorta and pulmonary artery expressed more receptors than saphenous vein or coronary artery. In this regard, the discovery of ghrelin receptors in the cardiovascular system opened a new research field on the role of ghrelin in the control of blood pressure and myocardial function (60,61).

3.1. Ghrelin acts as a cardioprotective hormone

The myocardium constitutes a source of ghrelin and obestatin (60), and GHS-R 1a is expressed in cardiomyocytes and other myocardial tissues (62), supporting the autocrine/paracrine effects of ghrelin in the heart. Acylated and desacyl ghrelin protect cardiomyocytes and endothelial cells against apoptosis through the activation of an intracellular survival pathway (63), whereas contradictory data have been reported regarding the effect of obestatin on cardiomyocyte viability (64, 65). Acylated ghrelin, des-Gln14-ghrelin and desacyl ghrelin show negative inotropic effects on papillary muscle by activating the release of cyclooxygenase metabolites in endocardial endothelium (66). Ghrelin improves the cachexia and the altered myocardial energy metabolism in rats with heart failure (67,68). In humans, plasma ghrelin and obestatin levels are increased in cachexia associated with chronic heart failure (CHF) (69, 70). The beneficial hemodynamic effects of ghrelin on patients with CHF seem to be attributable to both a positive inotropism of GH and a fall in cardiac overload. Furthermore, intravenous administration of
ghrelin in healthy individuals and patients with CHF improves the cardiac function by increasing the cardiac index and stroke volume index (71,72). Nevertheless, hearts of patients with CHF exhibit an impaired ghrelin production which might reflect maladaptive processes and a probably compensatory increase in GHS-R 1a expression (62).

Ghrelin exerts performance-enhancing effects on myocardial function. Chronic administration of ghrelin after myocardial infarction has been shown to attenuate LV enlargement and myocardial fibrosis by the suppression of cardiac sympathetic activity in rodents (67,73). The analysis by tissue Doppler imaging in healthy, young, normal-weight men with chronic intravenous administration of ghrelin showed an increase in the tissue tracking, which reflects an improvement in the global longitudinal systolic contraction of LV amplitude (74). Common variants in the human GHSR gene, which are associated with parameters of LV mass and geometry independently of blood pressure and BMI, have been identified in the general population (57). Moreover, a single-nucleotide polymorphism in the GHRL gene 5’ flanking area (-501A>C) associated with LV mass index after adjustments for age, sex and systolic blood pressure has been recently described (75). In this sense, circulating acylated ghrelin concentrations are increased in patients with LV hypertrophy compared with those with appropriate LV mass, which might reflect the LV remodelling during the progression to LV hypertrophy (46).

3.2. Hypotensive effect of ghrelin
Growing evidence supports that ghrelin reduces arterial pressure and systemic vascular resistance. Plasma ghrelin and obestatin levels are increased in hypertensive rats (76). Intracerebroventricular administration of ghrelin into the nucleus of the solitary tract (brain region involved in the regulation of cardiovascular system) induces a decrease in blood pressure, heart rate and also suppressed sympathetic renal activity in experimental animals (77,78). Intravenous administration of obestatin does not change blood pressure values of spontaneously hypertensive rats (SHR) (79). In humans, intravenous administration of ghrelin in healthy individuals and patients with CHF decreases mean arterial pressure, without changes in heart rate (69,70). In patients with the metabolic syndrome, high plasma concentrations of acylated ghrelin are associated with significantly higher blood pressure values, even after adjusting for BMI (46). Given the physiological relevance of acylated ghrelin, its increased production may represent a compensatory hypotensive mechanism.

Ghrelin causes vasorelaxation through different molecular mechanisms. Firstly, ghrelin improves endothelial dysfunction in GH-deficient rats by increasing endothelial nitric oxide synthase (eNOS) expression in the aorta, suggesting that this vascular effect is mediated by a GH-independent mechanism (80). Ghrelin directly stimulates the production of NO from vascular endothelial cells using PI3K-dependent signaling pathways that mimic those of insulin (81). In humans, intraarterial infusion of ghrelin induces vasodilation through GH/IGF-1-independent mechanisms in healthy individuals (82) and reverses endothelial dysfunction in patients with metabolic syndrome by increasing NO bioactivity (83). Secondly, ghrelin inhibits vascular oxidative stress through the inhibition of vascular NAD(P)H oxidases activity in SHR (84). In this regard, systemic administration of desacetyl ghrelin, but not acylated ghrelin, prevented diabetes-induced endothelial progenitor cell damage by modulating the NADPH oxidase regulatory protein Rac1 and improved the vasculogenic potential in individuals with type 2 diabetes (85). Ghrelin also inhibits proinflammatory cytokine production, mononuclear cell binding, and nuclear factor-xB (NFκB) activation in human endothelial cells in vitro, and hence, reduces the endothelial inflammation (86). Finally, ghrelin exerts an endothelium-independent depressor action in rat aorta by inhibiting angiotensin II-induced vasoconstriction (87). Furthermore, ghrelin constitutes an effective endothelium-independent vasodilator of the long-lasting constrictor endothelin-1 in human internal mammary arteries producing responses similar to those of adrenomedullin (88). Infusion of ghrelin in patients with metabolic syndrome restores the balance between vasoconstrictor endothelin-1 and vasodilator NO mediators (89). In summary, ghrelin increases NO bioavailability, prevents vascular oxidative stress and antagonizes the contraction of potent vasoconstrictor peptides, thereby preserving vascular homeostasis.

4. Conclusions
In summary, ghrelin exerts important cardiovascular actions in addition to its orexigenic and adipogenic effects, as shown in Figure 2. On the contrary, the contradictory observations of the role of obestatin in the viability of cardiomyocytes raise some questions regarding its involvement as a cardioprotective hormone (64,65). Further studies will be necessary to establish the specific contribution of obestatin in the myocardium. Likewise, further investigations in novel mutations, single-nucleotide polymorphisms or differential expression levels of molecules of the ghrelin system are needed to broaden our understanding of their role in the onset of obesity-associated CMD.
**Figure 2. Central and peripheral effects of ghrelin in the control of cardiovascular homeostasis.** By activating vagal afferents or via the bloodstream, ghrelin acts on the nucleus of the solitary tract inducing a decrease in blood pressure, heart rate and also a suppression of sympathetic renal activity. Cardiac effects of ghrelin include the inhibition of cardiomyocyte and endothelial cell apoptosis, the improvement of LV function and the reduction of fibrosis after myocardial injury, as well as the increase in cardiac index and stroke volume index after bolus injection of ghrelin. Vascular effects of ghrelin include the increase in NO bioavailability by increasing eNOS and by reducing the vascular NAD(P)H oxidases activity, the inhibition of cytokine release from blood vessels as well as the antagonism of Ang II and ET-1-induced vasoconstriction.

**Acknowledgments**

This work was funded by the Instituto de Salud Carlos III (FIS PS09/02330 and FIS PI10/01677). CIBER de Fisiopatología de la Obesidad y Nutrición (CIBERobn) is an initiative of the Instituto de Salud Carlos III, Spain.

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Review


