cardiovascular effects through GH-independent mechanisms.

3. Regulation of Ghrelin Secretion and the Circulating Ghrelin Level

Ghrelin is secreted from the stomach and circulates in the bloodstream.[40] The ghrelin level in the blood and mRNA levels in the stomach are increased by fasting and decreased by feeding.[43-47] Oral or intravenous administration of glucose is associated with a decrease in the plasma ghrelin level.[32,48] In addition, the plasma ghrelin level is decreased by ingestion of meals containing high concentrations of lipids and increased by meals low in protein.[49] Because gastric distention caused by water intake does not change the plasma ghrelin level, mechanical distention of the stomach cannot be the cause of ghrelin release.[32] The plasma ghrelin level is low in obese people and high in lean people.[50,51] Furthermore, the plasma ghrelin level is increased in patients with either bulimia nervosa[52] or anorexia nervosa,[53,54] but returns to basal levels following weight gain and recovery from the latter disease.[53,54]

To examine the pathophysiological significance of ghrelin in CHF, we determined the plasma ghrelin level in 74 patients with CHF (LV ejection fraction 28% ± 1%).[55] The plasma ghrelin level did not significantly differ between patients with CHF and control subjects; however, the plasma ghrelin level was significantly higher in patients with CHF who had cachexia than in those without cachexia (237 ± 18 vs 147 ± 10 fmol/mL, $p < 0.001$). Ghrelin stimulates secretion of GH, which is an anabolic hormone that is essential for skeletal and myocardial growth and metabolic homeostasis.[11,12] Recent studies have shown that peripheral administration of ghrelin induces weight gain by decreasing fat utilisation and increasing carbohydrate utilisation through a GH-independent mechanism.[32] In addition, ghrelin has been shown to elicit potent, long-lasting stimulation of food intake via activation of neuropeptide Y (NPY) neurons in the hypothalamic arcuate nucleus.[31,56] Thus, ghrelin causes a positive energy balance, not only by stimulating GH release, but also by stimulating food intake and decreasing fat utilisation through GH-independent mechanisms. These results suggest that increased plasma ghrelin levels may represent a compensatory mechanism under conditions of anabolic/catabolic imbalance in cachectic patients with CHF. As the majority of ghrelin is produced by X/A-like cells in the stomach,[39] it is interesting to speculate that the stomach has a role as an endocrine organ in the regulation of energy balance.

4. Biological Actions

The biological effects of ghrelin can be divided into GH-dependent effects and GH-independent effects, including orexigenic effects, inhibition of cell apoptosis, attenuation of sympathetic nerve activation, haemodynamic effects and gastrointestinal functions (figure 1b and figure 2).

4.1 GH-Releasing Activity

Ghrelin has been shown to increase GH release in a dose-dependent manner.[57] Intravenous injection of ghrelin markedly increased circulating GH levels in rats and humans, with greater potency than GHRH.[58] The peak level of GH occurred at 15–20

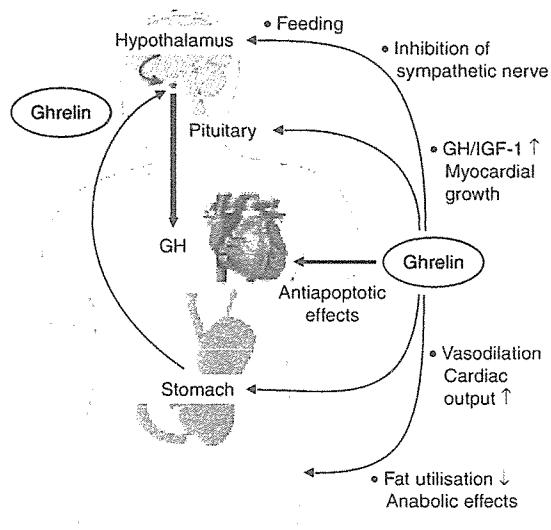

Fig. 2. A schematic illustration of various biological functions of ghrelin. **GH** = growth hormone; **IGF-1** = insulin-like growth factor-1.

minutes after a bolus of ghrelin, and the elevation of the GH level lasted for longer than 60 minutes.[17] These results suggest that ghrelin elicits potent, long-lasting GH release. A recent study demonstrated that blockade of the gastric vagal afferents attenuated ghrelin-induced feeding and GH secretion.[59] In addition, this study showed that ghrelin receptors were synthesised in vagal afferent neurons and transported to the afferent terminals. These findings suggest that gastric vagal afferents are the major pathway conveying ghrelin signals for GH secretion and starvation to the brain. Furthermore, recent studies[60-62] have demonstrated the interaction between the ghrelin/GHS-R and GHRH/GHRH receptor systems. Coadministration of ghrelin and GHRH has a synergistic effect on GH secretion.[60] Ghrelin regulates the production of GHRH via the hypothalamic GHS-R.[61] In particular, Gq/11 signaling is critically involved in the regulation of hypothalamic GHRH production.[62] Ghrelin potentiates GHRH-induced cAMP production in cells expressing GHRH and GHS-R, which may be attributable to direct interactions between the GHS-R and GHRH receptor.[63] These findings suggest that ghrelin stimulates GH release predominantly via activation of GHS-R but also partially via enhancement of the GHRH/GHRH receptor system (figure 1c).

4.2 Orexigenic Effects

Ghrelin is the first appetite-stimulatory peptide isolated from the stomach. The appetite-stimulatory nature of ghrelin is suggested by the findings that the ghrelin level in the blood and mRNA level in the stomach are increased and decreased by fasting and feeding, respectively,[43] and also that hyperglycaemia suppresses the circulating ghrelin level.[48] In fact, peripheral and intracerebroventricular administration of ghrelin stimulated food intake and increased body weight in normal rats and in GH-deficient dwarf rats.[31,32,64,65] The hypothalamic arcuate nucleus is the main active site of ghrelin. Hypothalamic NPY mRNA expression was increased in rats that received intracerebroventricular injection of ghrelin.[56] The orexigenic effect of ghrelin was abolished dose-dependently by co-injection with an NPY Y1 receptor antagonist. Interestingly, leptin-induced inhibition of food intake was reversed by co-injection of ghrelin in a dose-dependent manner.[56] In summary, ghrelin is an orexigenic peptide that antagonises the action of leptin through activation of the hypothalamic NPY/Y1 receptor pathway.

4.3 Inhibition of Cell Apoptosis

The endocrine activities of ghrelin are entirely dependent on its acylation and are mediated by the GHS-R. Des-acyl ghrelin, which is far more abundant than ghrelin, does not bind to the GHS-R, is devoid of any endocrine activity and its function is currently unknown. Recently, Baldanzi et al.[19] showed that both ghrelin and des-acyl ghrelin inhibit apoptosis of primary adult and H9c2 cardiomyocytes and endothelial cells *in vitro* through activation of extracellular signal-regulated kinase-1/2 and Akt serine kinases. In addition, ghrelin and des-acyl ghrelin recognise common high-affinity binding sites on H9c2 cardiomyocytes, which do not express GHS-R. Finally, both ibutamoren and examorelin, a nonpeptidyl and a peptidyl synthetic GHS, respectively, recognise the common ghrelin and des-acyl ghrelin binding sites, inhibit cell death, and activate mitogen-activated protein kinase and Akt. These findings provide the evidence that, independent of its acylation, the ghrelin gene product may directly act as a survival factor for the cardiovascular system through binding to a novel, yet to be identified receptor that is distinct from the GHS-R.

4.4 Attenuation of Sympathetic Nerve Activity

Microinjection of ghrelin into the nucleus of the solitary tract of rats and rabbits has been shown to suppress renal sympathetic nerve activity and significantly decrease mean arterial pressure and heart rate.[20,21] Pretreatment with an intravenous injection of pentolinium, a ganglion-blocking agent, eliminated these cardiovascular responses; however, pretreatment with an intravenous injection of atropine, an antagonist of muscarinic acetylcholine receptors, failed to prevent them.[21] Immunohistochemical

analysis revealed that the GHS-R was expressed in the neuronal cells of the nucleus of the solitary tract and the dorsal motor nucleus of the vagus nerve, but not in the cells of the area postrema. These results suggest that ghrelin acts at the nucleus of the solitary tract to suppress sympathetic activity and to decrease arterial pressure in rats and rabbits.

4.5 Acute Haemodynamic Effects

GHS-R mRNA is detectable in the heart and blood vessels in rats and humans.[17,66] To clarify whether ghrelin has direct vasodilatory effects in humans, the response of forearm bloodflow to intra-arterial infusion of ghrelin was examined using a plethysmograph. Using this technique, ghrelin was seen to increase forearm blood flow in a dose-dependent manner.[67] A single injection of ghrelin significantly decreased mean arterial pressure in rats both with and without CHF.[68] This hypotensive effect was also observed in GH-deficient rats. Interestingly, Wiley and Davenport[69] have demonstrated that ghrelin induces vasodilation in isolated human endothelium-denuded arteries. This result suggests that ghrelin is an endothelium-independent vasodilator. In patients with CHF, intravenous infusion of human ghrelin significantly decreased mean arterial pressure without a significant change in heart rate.[70] The hypotensive effect of ghrelin may be explained by its direct vasodilatory effect[68,69] and inhibition of sympathetic nerve activity.[20,21] Ghrelin significantly increased cardiac index (+25%, $p < 0.05$) and stroke volume index (+30%, $p < 0.05$) in patients with CHF. *In vitro*, fractional cell shortening was not significantly altered by 1, 10 and 10 Pmol/mL doses of ghrelin, suggesting that ghrelin has no direct inotropic effect. Thus, the increase in cardiac index may be attributable to a fall in cardiac afterload and an inotropic effect of GH. Infusion of ghrelin did not significantly alter urine volume, urinary sodium excretion or creatinine clearance.[70] These results suggest that intravenous infusion of ghrelin, a potent GH-releasing peptide, had beneficial haemodynamic effects in patients with CHF in the absence of renal effects.

4.6 Chronic Haemodynamic Effects

GH and IGF-1 are essential for skeletal and myocardial growth.[11,12] Earlier studies have shown that GH supplementation may have beneficial effects on myocardial structure and function in some patients with CHF.[14-16] Treatment with ghrelin 100 μg/kg twice daily for 3 weeks significantly increased circulating IGF-1 levels in rats with CHF.[68] Repeated administration of ghrelin increased posterior wall thickness, inhibited progressive LV enlargement and, thereby, reduced LV wall stress.[68] These findings suggest that ghrelin improves cardiac structure, at least in part through GH/IGF-1-dependent mechanisms.

4.7 Gastrointestinal Functions

Intravenous administration of ghrelin increases gastric acid secretion and stimulates gastric motility.[71,72] The maximum gastric acid response to ghrelin is almost as high as that elicited by subcutaneous treatment with histamine. These responses to ghrelin can be abolished by pretreatment with either atropine or bilateral cervical vagotomy, but not by pretreatment with a histamine H_2-receptor antagonist. Intracerebroventricular administration of ghrelin also increases gastric acid secretion[73] and induces c-fos expression in the nucleus of the solitary tract and the dorsomotor nucleus of the vagus nerve. These findings indicate that the ability of ghrelin to stimulate gastric acid secretion is mediated by activation of the vagus nerve. **((Author: which references for this section if 71-73 are to be deleted?))**

5. Therapeutic Potential of Ghrelin in Treatment of Chronic Heart Failure

Both ventricular dysfunction and cachectic conditions are therapeutic targets in CHF. A variety of GH-dependent and -independent actions of ghrelin indicate its therapeutic potential in the treatment of CHF. This section focuses on the effects of repeated administration of ghrelin on cardiac function and cardiac cachexia in CHF.

5.1 Improvement in Cardiac Function

As GH and IGF-1, are essential for skeletal and myocardial growth and metabolic homeostasis,[11,12] and earlier studies had shown that GH supplementation may have beneficial effects on myocardial structure and function, **((Author: which references here? Or should we add (see sections 4.5 and 4.6) or similar?))** we investigated the effects of ghrelin on LV function, exercise capacity and muscle wasting in patients with CHF.[74] Human synthetic ghrelin 2 μg/kg twice daily was intravenously administered to patients with CHF for 3 weeks. Ghrelin increased LV ejection fraction in association with an increase in LV mass and a decrease in LV end-systolic volume (both $p < 0.05$). Treatment with ghrelin increased peak workload and peak oxygen consumption during exercise (both $p < 0.05$). These preliminary results suggest that repeated administration of ghrelin improves LV function and exercise capacity in patients with CHF. As reported in section 4.6, ghrelin also increased posterior wall thickness, inhibited progressive LV enlargement and, thereby, reduced LV wall stress in rats with CHF.[68] GH and IGF-1 have been shown to enhance physiological compensatory hypertrophy in rats with CHF, resulting in a decrease in LV wall stress, which in turn leads to an improvement in cardiac function.[75] Thus, ghrelin may also improve cardiac function through GH-dependent mechanisms. On the other hand, ghrelin inhibits apoptosis of cardiomyocytes and endothelial cells through activation of extracellular signal-regulated kinase-1/2 and Akt serine kinases,[19] implying that improvement in cardiac function may be related to direct effects of ghrelin on myocardium. Importantly, ghrelin significantly decreased plasma norepinephrine in patients with CHF.[74] Thus, the inhibitory effects of ghrelin on sympathetic nerve activity[20,21] may contribute to a decrease in plasma norepinephrine, which may have beneficial effects on cardiac performance in patients with CHF. Further studies are necessary to determine which actions of ghrelin are major contributors to improved cardiac function.

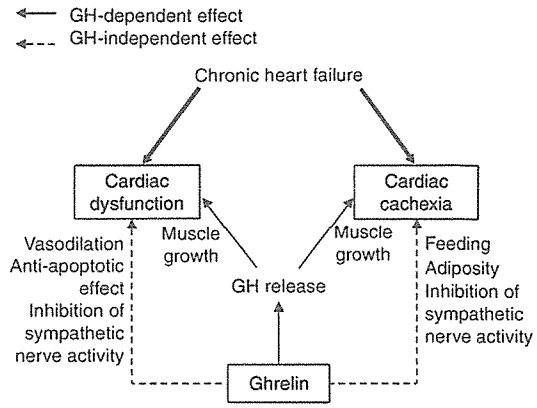

Fig. 3. Characteristics of end-stage heart failure and therapeutic effects of ghrelin. Left ventricular (LV) dysfunction and cardiac cachexia are often observed in patients with severe heart failure. Ghrelin improves LV dysfunction and attenuates the development of cardiac cachexia through a growth hormone (GH)-dependent mechanism and GH-independent mechanisms: inhibition of sympathetic nerve activity, stimulation of feeding, adiposity, vasodilation and anti-apoptotic effects.

5.2 Attenuation of Cardiac Cachexia

In cachetic patients, 3 weeks of administration of ghrelin tended to increase bodyweight and significantly increased lean body mass.[74] It also increased bodyweight, lean body mass and respiratory muscle strength in cachectic patients with chronic obstructive pulmonary disease.[75] These results suggest that ghrelin may have beneficial effects on cachexia. Considering ghrelin-stimulated GH release, these effects may be mediated, at least in part, by GH/IGF-1, which is essential for muscle growth. Earlier studies have shown that ghrelin induces orexigenic effects via activation of NPY neurons in the hypothalamic arcuate nucleus,[31,56] and that intravenous administration of ghrelin increased food intake in patients with CHF, which may contribute to anabolic effects of ghrelin.[74] Although many animal studies have documented beneficial effects of GH in CHF,[76] controlled studies in humans have not shown a benefit with treatment.[77,78] Nevertheless, ghrelin may have additional therapeutic potential because it has GH-independent effects such as attenuation of sympathetic nerve activities, vasodilatory actions, inhibition of cell apoptosis and orexigenic effects. Thus, administration of ghrelin may be a

new therapeutic approach to the treatment of cardiac cachexia (figure 3).

6. Conclusions

Ghrelin has cardiovascular effects and regulates energy metabolism through GH-dependent and GH-independent mechanisms. Exogenously administered ghrelin improves LV dysfunction and attenuates the development of cardiac cachexia in CHF. Thus, supplementation of ghrelin may be a new therapeutic approach to the treatment of CHF. However, large-scale, double-blind, randomised, placebo-controlled studies are needed to confirm this.

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INTERNAL MEDICINE

REVIEW ARTICLE

Ghrelin, a Novel Growth Hormone-releasing Peptide, in the Treatment of Cardiopulmonary-associated Cachexia

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Abstract

Ghrelin is a novel growth hormone (GH)-releasing peptide, isolated from the stomach, which has been identified as an endogenous ligand for GH secretagogue receptor. The discovery of ghrelin indicates that the release of GH from the pituitary might be regulated not only by hypothalamic GH-releasing hormone, but also by ghrelin derived from the stomach. This peptide also stimulates food intake and induces adiposity through GH-independent mechanisms. In addition, ghrelin acts directly on the central nervous system to decrease sympathetic nerve activity. Thus, ghrelin plays important roles for maintaining GH release and energy homeostasis. Repeated administration of ghrelin improves body composition, muscle wasting, functional capacity, and sympathetic augumentation in cachectic patients with heart failure or chronic obstructive pulmonary disease. These results suggest that ghrelin has anti-cachectic effects through GH-dependent and independent mechanisms. Thus, administration of ghrelin may be a new therapeutic strategy for the treatment of cardiopulmonary-associated cachexia.

Key words: heart failure, chronic obstructive pulmonary disease, growth hormone, feeding

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Introduction

Small synthetic molecules called growth hormone secretagogues (GHSs) stimulate the release of growth hormone (GH) from the pituitary (1-3). They act through the GH secretagogue receptor (GHS-R), a G protein-coupled receptor with seven transmembrane domains (4). Using a reverse pharmacology paradigm with a stable cell line expressing GHS-R, Kojima et al purified an endogenous ligand for GHS-R from rat stomach and named it ghrelin (5). Ghrelin is a peptide hormone in which the third amino acid, usually a serine but in some species a threonine, is modified by a fatty acid; this modification is essential for ghrelin's activity. The discovery of ghrelin indicates that the release of GH from the pituitary might be regulated not only by hypothalamic GH-releasing hormone, but also by ghrelin derived from the stomach. In addition, ghrelin stimulates appetite by acting on the hypothalamic arcuate nucleus (6, 7). It is secreted from the stomach and circulates in the bloodstream under fasting conditions, indicating that it transmits a hunger signal from the periphery to the central nervous system (8). Taking into account all these activities, ghrelin plays important roles for maintaining GH release and energy homeostasis.

GH and its mediator, insulin-like growth factor-1 (IGF-1), are anabolic hormones that are essential for skeletal and myocardial growth and metabolic homeostasis (9, 10). Considering the anabolic effects of GH/IGF-1, ghrelin may have beneficial effects on functional capacity and energy metabolism in cardiopulmonary-associated cachexia through GH-dependent mechanisms. On the other hand, ghrelin may have direct metabolic effects through GH-independent mechanisms: orexigenic effects (6, 7); attenuation of fat utilization (11); and inhibition of sympathetic nerve activities (12, 13). Taking these physiological effects together, ghrelin causes a positive energy balance through GH-dependent and -independent mechanisms. Cachexia, which is a catabolic state characterized by weight loss and muscle wasting, is associated with hormonal changes and cytokine

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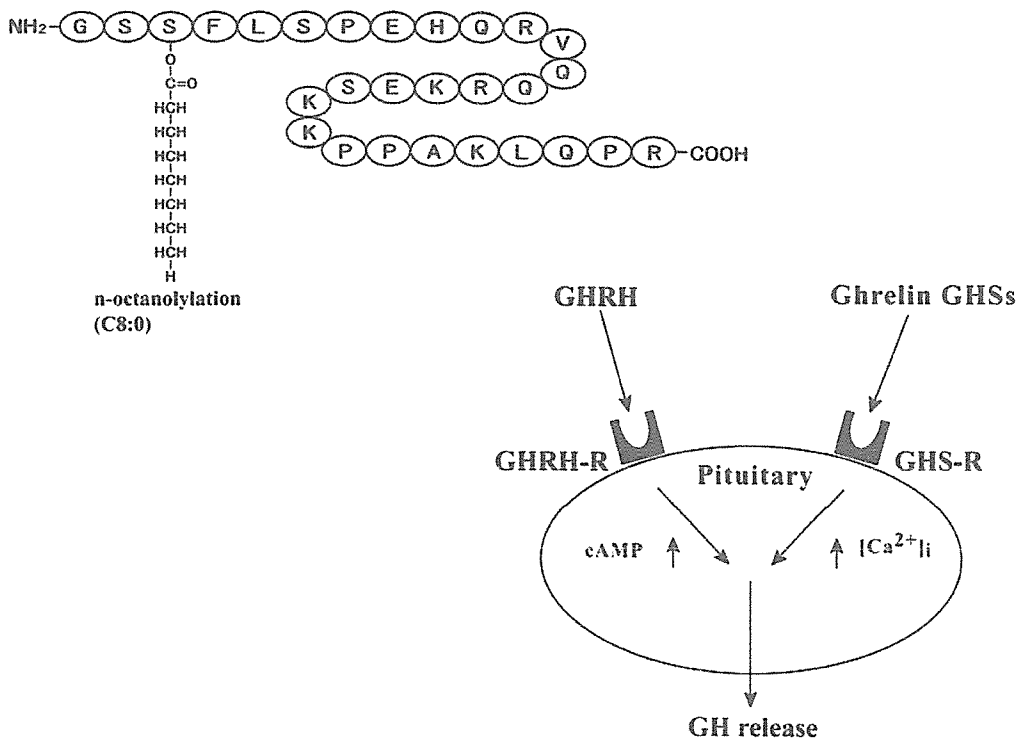

Figure 1. **A, Structure of human ghrelin. Human ghrelin is a 28-amino-acid peptide containing an n-octanoyl modification. B, Stimulation of GH release by GHRH and ghrelin. GHRH acts on the GHRH receptor through a cAMP-dependent mechanism, whereas ghrelin and GHSs bind to the GHS receptor, followed by the release of Ca from intracellular stores.**

activation (14-17). Importantly, the presence of cachexia is a strong independent risk factor for mortality in a variety of diseases such as chronic heart failure (CHF) (18) or chronic obstructive pulmonary disease (COPD) (19). Thus, cachexia is a therapeutic target in the treatment of such patients. This article summarizes the physiological functions of ghrelin and its therapeutic potential for the treatment of cardiopulmonary-associated cachexia.

GHSs and discovery of ghrelin

In addition to the physiological stimulation by GH-releasing hormone (GHRH), release of GH from the pituitary is stimulated by small synthetic molecules called GHSs (1-3). They act through GHS-R, a G-protein-coupled receptor (4), for which the ligand was unknown until the discovery of ghrelin. GHSs are synthetic peptidyl and non-peptidyl molecules that have strong, dose-dependent GH-releasing activity *in vivo*. GHSs are a heterogenous group, but they have the following common characteristics. GHSs are synthetic substances, stimulate GH release from the pituitary, and act through the GHS-R, but not the GHRH receptor. The GHS family includes peptidyl molecules such as GHRP-6 and hexarelin and nonpeptidyle molecules such as L-692429 and MK-0677. In 1995, Merck Co. discoverd MK-0677 which has bioavailability and long-lasting effects after oral administration (20). These GHS compounds have entered clinical trials. Potential therapeutic indications for GHSs include idiopathic GH deficiency states, stimulation of processes in the elderly, and supportive therapy in catabolic wasting conditions. GHRH acts on the GHRH receptor through a cAMP-dependent mechanism. On the other hand, GHSs bind to the GHS-R and activate phospholipase C, leading to increased inositol phosphate turnover and protein kinase C activation, followed by the release of Ca^{2+} from intracellular stores. Using GHS-R-expressing cells to monitor intracellular Ca^{2+} concentration, we found that GHS-R was activated by stomach extracts. Thus, an endogenous ligand specific for GHS-R, ghrelin, was successfully isolated from the stomach in December 1999 (5). Human ghrelin is a 28-amino-acid peptide containing an n-octanoyl modification at serine 3 and is homologous to rat ghrelin apart from two amino acids (Fig. 1). The n-octanolylation at ser3 is considered to be essential for the activity of ghrelin.

Production and distribution of ghrelin and its receptor

Ghrelin is produced mainly in the stomach. To date, four types of endocrine cells, ECL, D, enterochromaffin, and X/A-like cells have been identified in the oxyntic mucosa of the stomach (21). Date et al have reported that X/A-like

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cells, whose hormonal product has not previously been clarified, secrete ghrelin (22). Ghrelin is not secreted into the gastrointestinal tract, but is secreted into blood vessels. Thus, the plasma ghrelin level is relatively high (100-120 fmol/ml) (23). The plasma ghrelin level falls markedly following gastrectomy (24, 25). Ghrelin is also produced in the small and large intestines and is detected in a limited region of the hypothalamic arcuate nucleus which is involved in the regulation of food intake (5). These results suggest that ghrelin serves as a circulating factor as well as an autocrine/paracrine factor. Before the discovery of the endogenous ligand, a specific receptor for ghrelin, GHS-R, was discovered in 1996 using an expression cloning strategy (4). It is a G-protein-coupled receptor with seven transmembrane domains. GHS-R is present in a variety of tissues including the pituitary and hypothalamus, and is distinct from the GHRH receptor. Interestingly, GHS-R is detected in the cardiac ventricles and blood vessels, suggesting that ghrelin may cause cardiovascular effects through GH-independent mechanisms.

Physiological functions

(1) GH-releasing activity

Ghrelin stimulates GH release both *in vitro* and *in vivo*. Intravenous injection of ghrelin dose dependently increases circulating GH in rats (5) and humans (26), with greater potency than GHRH (27). The peak level of GH occurred at 15-20 min after a bolus of ghrelin, and the elevation of GH level lasted longer than 60 min after bolus injection (28). These results suggest that ghrelin elicits a potent, long-lasting GH release. A recent study demonstrated that blockade of the gastric vagal afferents attenuates ghrelin-induced GH secretion and completely abolishes feeding (29). In addition, ghrelin receptors are synthesized in vagal afferent neurons and transported to the afferent terminals. These findings suggest that gastric vagal afferents are the major pathway conveying ghrelin's signals for GH secretion and starvation to the brain. Furthermore, recent studies have demonstrated the interaction between the ghrelin/GHS-R and GHRH/GHRH receptor systems. Coadministration of ghrelin and GHRH has a synergistic effect on GH secretion, and their combined administration is the most potent inducer of GH release yet identified (30). Ghrelin regulates the production of GHRH via the hypothalamic GHS-R (31). Particularly, Gq/11 signaling is critically involved in the regulation of hypothalamic GHRH production (32). Ghrelin potentiates GHRH-induced cAMP production in cells expressing GHRH and GHS-R, which may be attributable to direct interactions between the GHS-R and GHRH receptor (33). Further studies are necessary to elucidate the synergetic effects of ghrelin and GHRH on the pituitary hormone axis.

(2) Orexigenic effect

Ghrelin is the first appetite-stimulatory peptide isolated from the stomach. The ghrelin level in the blood and mRNA level in the stomach are increased by fasting and decreased by feeding (8). In addition, hyperglycaemia suppresses the circulating ghrelin level (34). These results suggest that ghrelin serves as an appetite-stimulatory peptide. In fact, peripheral and intracerebroventricular administration of ghrelin stimulates food intake and increases body weight in normal rats and in GH-deficient dwarf rats (6, 11, 35, 36). The hypothalamic arcuate nucleus is the main active site of ghrelin. Hypothalamic neuropeptide Y (NPY) mRNA expression was increased in rats that received intracerebroventricular injection of ghrelin (7). Ghrelin's orexigenic effect was abolished dose dependently by co-injection of NPY Y1 receptor antagonist. Thus, ghrelin elicits potent stimulation of food intake via activation of NPY neurones in the hypothalamic arcuate nucleus. Interestingly, leptin-induced inhibition of food intake was reversed by co-injection of ghrelin in a dose-dependent manner (7). Taking these findings together, ghrelin is an orexigenic peptide that antagonizes the action of leptin through activation of the hypothalamic NPY Y1 receptor pathway.

(3) Hemodynamic effect

GHS-R mRNA is detectable in the heart and blood vessels in rats and humans (28, 37). To clarify whether ghrelin has direct vasodilatory effects in humans, the response of forearm blood flow to intra-arterial infusion of ghrelin was examined using a plethysmograph. Ghrelin increased forearm blood flow in a dose-dependent manner (38). A single injection of ghrelin significantly decreased mean arterial pressure both in sham-operated rats and CHF rats (39). This hypotensive effect was also observed in GH-deficient rats. Interestingly, Wiley Davenport have demonstrated that ghrelin is an endothelium-independent vasodilator of the long-lasting constrictor endothelin-1 in isolated human arteries (40). These results suggest that ghrelin has direct vasodilatory effects. In patients with CHF, intravenous infusion of human ghrelin significantly decreased the mean arterial pressure without a significant change in heart rate (41). Ghrelin significantly increased the cardiac index and stroke volume index. *In vitro*, fractional cell shortening was not significantly altered by each dose of ghrelin, suggesting that ghrelin has no direct inotropic effect.

(4) Inhibition of cell apoptosis

Ghrelin endocrine activities are entirely dependent on its acylation and are mediated by GHS-R. Des-acyl ghrelin, which is far more abundant than ghrelin, does not bind GHS-R, is devoid of any endocrine activity, and its function is currently unknown. Recently, Baldanzi et al showed that both ghrelin and des-acyl ghrelin inhibit apoptosis of primary adult and H9c2 cardiomyocytes and endothelial cells in vitro through activation of extracellular signal-regulated kinase-1/2 and Akt serine kinases (42). In addition, ghrelin and des-acyl ghrelin recognize common high affinity binding sites on H9c2 cardiomyocytes, which do not express GHS-R. Finally, both MK-0677 and hexarelin, a nonpeptidyl and a peptidyl synthetic GHS, respectively, recognize the common ghrelin and des-acyl ghrelin binding sites, inhibit cell death, and activate MAPK and Akt. These findings provide the evidence that, independent of its acylation, the ghrelin gene product may act as a survival factor directly on

the cardiovascular system through binding to a novel, yet to be identified receptor, which is distinct from GHS-R.

(5) Attenuation of sympathetic nerve activity

Microinjection of ghrelin into the nucleus of the solitary tract suppressed the renal sympathetic nerve activity and significantly decreased the mean arterial pressure and heart rate (12, 13). Pretreatment with intravenous injection of pentolinium, a ganglion-blocking agent, eliminated these cardiovascular responses induced by the microinjection of ghrelin into the nucleus of the solitary tract; however, pretreatment with intravenous injection of atropine sulfate, an antagonist of muscarinic acetylcholine receptors, failed to prevent them. Immunohistochemical study revealed that GHS-R, the receptor for ghrelin, was expressed in the neuronal cells of the nucleus of the solitary tract and the dorsal motor nucleus of the vagus, but not in the cells of the area postrema. These results suggest that ghrelin acts at the nucleus of the solitary tract to suppress sympathetic activity and to decrease arterial pressure in rats.

(6) Gastrointestinal functions

Intravenous administration of ghrelin increases gastric acid secretion and stimulates gastric motility (43, 44). The maximum response to ghrelin is almost as high as that elicited by subcutaneous treatment with histamine. These responses to ghrelin were abolished by pretreatment with either atropine or bilateral cervical vagotomy, but not by a histamine H2-receptor antagonist. Intracerebroventricular administration of ghrelin also increases gastric acid secretion (45) and induces c-fos expression in the nucleus of the solitary tract and the dorsomotor nucleus of the vagus nerve. These findings indicate that ghrelin's ability to stimulate gastric acid secretion is mediated by activation of the vagus nerve.

Regulation of ghrelin secretion and circulating level

Ghrelin is secreted from the stomach and circulates in the bloodstream. Ghrelin level in the blood and mRNA in the stomach are increased by fasting and decreased by feeding (8, 46-48). Oral or intravenous administration of glucose decreases plasma ghrelin level (11, 34). In addition, the plasma ghrelin level is decreased by meals containing high concentrations of lipids and increased by meals low in protein (49). Because gastric distention caused by water intake does not change the plasma ghrelin level, mechanical distention of the stomach cannot be the cause of ghrelin release (11). Plasma ghrelin level is low in obese people and high in lean people (50, 51). Furthermore, the plasma ghrelin level is increased in both bulima nervosa and anorexia nervosa patients, but in the case of the latter, it returns to basal levels following weight gain and recovery from the disease (52-54). Patients with CHF or COPD often show a certain degree of cachexia. Cachexia is an independent risk factor for mortality in such patients. The plasma ghrelin level was significantly higher in CHF patients with cachexia than in those without cachexia, although the plasma ghrelin level did not significantly differ between CHF patients and control subjects (55). Similarly, plasma ghrelin was elevated in underweight patients with COPD, and the level was associated with a cachectic state and abnormality of pulmonary function (56). Considering the anabolic effects of ghrelin, increased plasma ghrelin may represent a compensatory mechanism under conditions of anabolic/catabolic imbalance in cachectic patients with CHF or COPD. The majority of ghrelin is produced by X/A-like cells in the stomach (22), although a small amount of ghrelin is produced by the arcuate nucleus of the hypothalamus. Thus, it is interesting to speculate that the stomach has a role as an endocrine organ in the regulation of energy balance.

Feature without ghrelin

The stomach is the major source of circulating ghrelin. Total gastrectomy, as is performed in the treatment of gastric cancer or severe gastric ulcers, decreases the plasma concentrations of ghrelin to 30–50% of the baseline value when measured at 30 min after the operation (24, 25). This concentration gradually increased to 70% of the level before the operation. These results indicate that gastric ghrelin production accounts for more than half of circulating ghrelin. Gastric factors are considered to control bone formation (57) because total gastrectomy sometimes induces osteopenia (58). Synthetic ghrelin agonists, GHSs, have been shown to directly stimulate osteocyte growth (59). Thus, ghrelin may be involved in the gastric regulation of bone formation. Surprisingly, ghrelin knockout mice showed normal size, growth rate, food intake, body composition, reproduction, and gross behavior, without any pathological changes (60, 61). However, the ghrelin-null mouse showed a significant reduction in respiratory quotient and a trend for lower body fat mass when the mouse was fed with a high-fat diet (61). These results indicate that ghrelin may function in nutrient sensing and switching of metabolic substrates. Mice lacking GHS-R do not show the typical increases in GH release and food intake upon ghrelin administration (62), indicating that GHS-R is indeed the primary biologically relevant ghrelin receptor. Although exogenously administered ghrelin is known to induce growth and food intake, genetic inactivation of the ghrelin gene does not affect growth or food intake in mice (60, 61). This could be explained by activation of a compensatory pathway in the mutant mice. On the other hand, a small interfering RNA-based knockdown of the GHS-R in rats impairs somatic growth, weight gain, and food intake (63). These findings suggest that the GHS-R is more important for growth and food intake than ghrelin itself.

Clinical application of ghrelin

(1) Cardiac cachexia

LV dysfunction and remodeling and cardiac cachexia are often observed in patients with end-stage CHF (64). GH and its mediator, IGF-1, are essential for skeletal and myocardial growth and metabolic homeostasis (9, 10). Earlier studies

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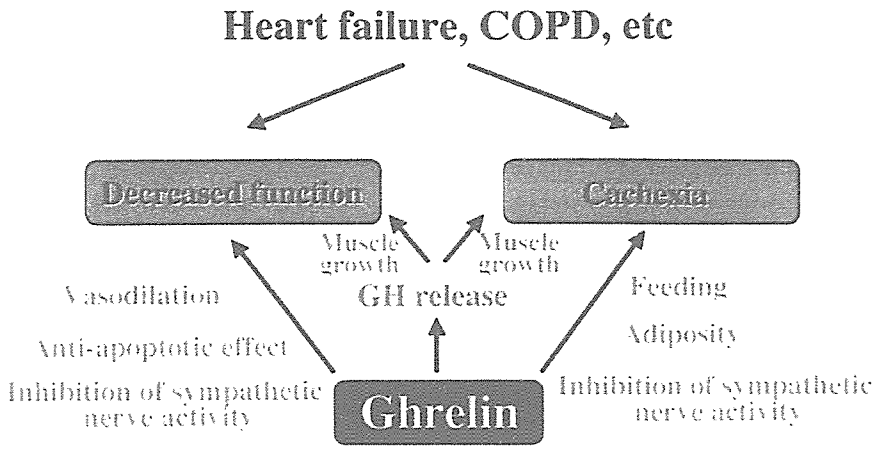

Figure 2. **Characteristics of end-stage CHF and COPD and the therapeutic potential of ghrelin. Ghrelin may increase the functional capacity and attenuate the development of cardiopulmonary-associated cachexia through GH-dependent and GH-independent mechanisms.**

have shown that GH supplementation may have beneficial effects on myocardial structure and function in some patients with CHF (65-67). Thus, we investigated the effects of ghrelin on left ventricular (LV) function, exercise capacity, and muscle wasting in patients with CHF (68). Human synthetic ghrelin (2 μg/kg twice a day) was intravenously administered to patients with CHF for three weeks. Ghrelin increased LV ejection fraction in association with an increase in LV mass and a decrease in LV end-systolic volume. Treatment with ghrelin increased peak workload and peak oxygen consumption during exercise. Ghrelin improved muscle wasting, as indicated by increases in muscle strength and lean body mass. These preliminary results suggest that repeated administration of ghrelin improves LV function, exercise capacity, and muscle wasting in patients with CHF. Ghrelin increased posterior wall thickness, inhibited progressive LV enlargement, and thereby reduced LV wall stress in rats with CHF (39). GH and its mediator, IGF-1, have been shown to enhance physiological compensatory hypertrophy in rats with CHF, resulting in a decrease in LV wall stress, leading to improvement in cardiac function (69). Thus, ghrelin may also improve cardiac function partly through GH-dependent mechanisms. On the other hand, ghrelin inhibits apoptosis of cardiomyocytes and endothelial cells through activation of extracellular signal-regulated kinase-1/2 and Akt serine kinases (42), implying that improvement in cardiac function may be related to the direct effects of ghrelin on myocardium. Importantly, ghrelin significantly decreased plasma norepinephrine in patients with CHF. A recent study has demonstrated that ghrelin acts directly on the central nervous system to decrease sympathetic nerve activity (12, 13). Thus, the inhibitory effects of ghrelin on sympathetic nerve activity may contribute to a decrease in plasma norepinephrine, which may have beneficial effects on cardiac performance in patients with CHF.

Cardiac cachexia, which is a catabolic state characterized by weight loss and muscle wasting, occurs frequently in patients with end-stage CHF (14-16) and is a strong independent risk factor for mortality in patients with CHF (18). Thus, cardiac cachexia may be a therapeutic target in the treatment of heart failure. Three-week administration of ghrelin tended to increase body weight and significantly increased lean body mass and muscle strength. These results suggest that treatment with ghrelin improves muscle wasting in patients with CHF. These effects may be mediated, at least in part, by GH/IGF-1, which is considered to be essential for skeletal muscle. Earlier studies have shown that ghrelin induces orexigenic effects via activation of neuropeptide Y neurons in the hypothalamic arcuate nucleus (6, 7). Intravenous administration of ghrelin increased food intake in patients with CHF, which may contribute to the anabolic effects of ghrelin. Although many animal studies have documented the beneficial effects of GH (65, 66, 69), controlled studies in humans have been predominantly negative (70, 71). Nevertheless, ghrelin may have additional therapeutic potential because it has GH-independent effects such as attenuation of sympathetic nerve activities, vasodilatory actions, inhibition of cell apoptosis, and orexigenic effects. Thus, administration of ghrelin may be a new therapeutic approach to the treatment of cardiac cachexia (Fig. 2).

(2) Pulmonary cachexia

Patients with COPD often show a certain degree of cachexia (17, 72). Cachexia is an independent risk factor for mortality in COPD (19, 73). GH treatment has been shown to increase muscle mass in patients with COPD (74). These findings suggest a role of the GH/IGF-1 axis in cachexia associated with COPD. Considering the anabolic effects of ghrelin, we investigated whether ghrelin improves cachexia and functional capacity in cachectic patients with COPD (75). Human ghrelin (2 μg/kg twice a day) was intravenously administered to cachectic patients with COPD for three weeks. Treatment with ghrelin resulted in a significant

increase in body weight. Food intake was significantly increased during ghrelin therapy. Ghrelin increased lean body mass, and peripheral and respiratory muscle strength. Ghrelin significantly increased Karnofsky performance status score and the distance walked in 6 min, although it did not significantly alter pulmonary function. Ghrelin attenuated the exaggerated sympathetic nerve activity, as indicated by a marked decrease in plasma norepinephrine level. These preliminary results suggest that repeated administration of ghrelin improves body composition, muscle wasting, functional capacity, and sympathetic augumentation in cachectic patients with COPD. Thus, administration of ghrelin may be a new therapeutic approach to the treatment of pulmonary cachexia (Fig. 2).

Additional clinical applications of ghrelin

(1) Various catabolic states

Other potential clinical applications of ghrelin are in osteoporosis, aging, and catabolic states including those seen in postoperative patients and in AIDS- and cancer-associated wasting syndromes (76-78).

(2) Eating disorder

Ghrelin may serve as an orexigenic agent for the treatment of eating disorders such as anorexia nervosa (79). Injection of ghrelin can stimulate appetite and improve the nutritional state of these patients. However, the plasma ghrelin level in anorexia nervosa is very high. This result indicates that sensitivity to ghrelin is severely disturbed in these individuals.

(3) Gastrointestinal disease

Ghrelin stimulates gastric motility (43, 44), which makes it a candidate for the treatment of postoperative gastric ileus. Ghrelin administration has been shown to have a strong prokinetic effect, accelerating gastric emptying and the small intestinal transit of liquid meals and reversing delayed gastric evacuation, thus counteracting gastric ileus (80).

(4) GH deficiency

Intravenous injection of ghrelin dose-dependently increases circulating GH in humans (26), with greater potency than GHRH (27). In addition, coadministration of ghrelin and GHRH has a synergistic effect on GH secretion, and their combined administration is the most potent inducer of GH release yet identified (30). Thus, supplementation with ghrelin may have beneficial effects on adult and child GH deficiency.

(5) Diagnosis of pituitary function

Because of its potent GH-releasing activity and specificity, ghrelin may be applied to the diagnosis and treatment of GH deficiency (81-83). To diagnose GH deficiency, the most common GH stimulus used is insulin-induced hypoglycemia, in which the blood glucose level is decreased to < 40 mg/dl. The hypoglycemic action of insulin may sometimes cause side effects. Intravenous injection of ghrelin in humans does not show any side effects, suggesting that ghrelin may be useful for diagnosing GH deficiency.

Conclusions

Ghrelin is a novel GH-releasing peptide, isolated from the stomach, which has been identified as an endogenous ligand for GHS-R. This peptide also has a variety of GH-independent effects such as orexigenic effects, attenuation of sympathetic nerve activities, vasodilatory actions, and inhibition of cell apoptosis. Repeated administration of ghrelin improves body composition, muscle wasting, functional capacity, and sympathetic augumentation in cachectic patients with CHF or COPD. These beneficial effects are mediated by both GH-dependent and -independent mechanisms. Thus, supplementation of ghrelin may be a new therapeutic approach for the treatment of cardiopulmonary-associated cachexia.

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