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Therapeutic Potential of Ghrelin in the Treatment of Heart Failure

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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 the GH secretagogues 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. Considering the haemodynamic and anabolic effects of GH, ghrelin may have beneficial effects on cardiac function and energy metabolism in heart failure through GH-dependent mechanisms. On the other hand, ghrelin has some GH-independent actions: ghrelin stimulates food intake and induces adiposity. Interestingly, ghrelin acts directly on the CNS to decrease sympathetic nerve activity. It also inhibits apoptosis of cardiomyocytes and endothelial cells. An experimental study has shown that repeated administration of ghrelin improves cardiac structure and function, and attenuates the development of cardiac cachexia in chronic heart failure (CHF). These results suggest that ghrelin has cardiovascular effects and regulates energy metabolism through GH-dependent and -independent mechanisms. Thus, administration of ghrelin may be a new therapeutic strategy for the treatment of severe CHF.

Heart failure is a major public health concern. Currently, there are 5 million Americans with congestive heart failure, with nearly 500 000 new cases every year.[1] In the past 10 years, several large-scale, randomised clinical trials have shown that ACE inhibitors and β-adrenoceptor antagonists (β-blockers) reduce the risk of death in patients with chronic heart failure (CHF).[2-9] Nevertheless, heart failure contributes to >250 000 deaths every year.

Ghrelin is a novel growth hormone (GH)-releasing peptide, isolated from the stomach, which has been identified as an endogenous ligand for the GH secretagogues (GHS) receptor (GHS-R).[10] Human ghrelin is a 28-amino-acid peptide containing an n-octanoyl modification at serine 3 (figure 1a). Ghrelin stimulates GH secretion through a mechanism independent of hypothalamic GH-releasing hormone (GHRH). The biological actions of ghrelin are divided into GH-dependent effects and GH-independent effects (figure 1b). GH and its mediator, insulin-like growth factor (IGF)-1, are anabolic hormones that are involved in several physiological processes, such as the control of muscle mass and function, body composition and regulation of nutrient metabolism.[11,12] In particular, the roles of GH and IGF-1 as modulators of myocardial structure

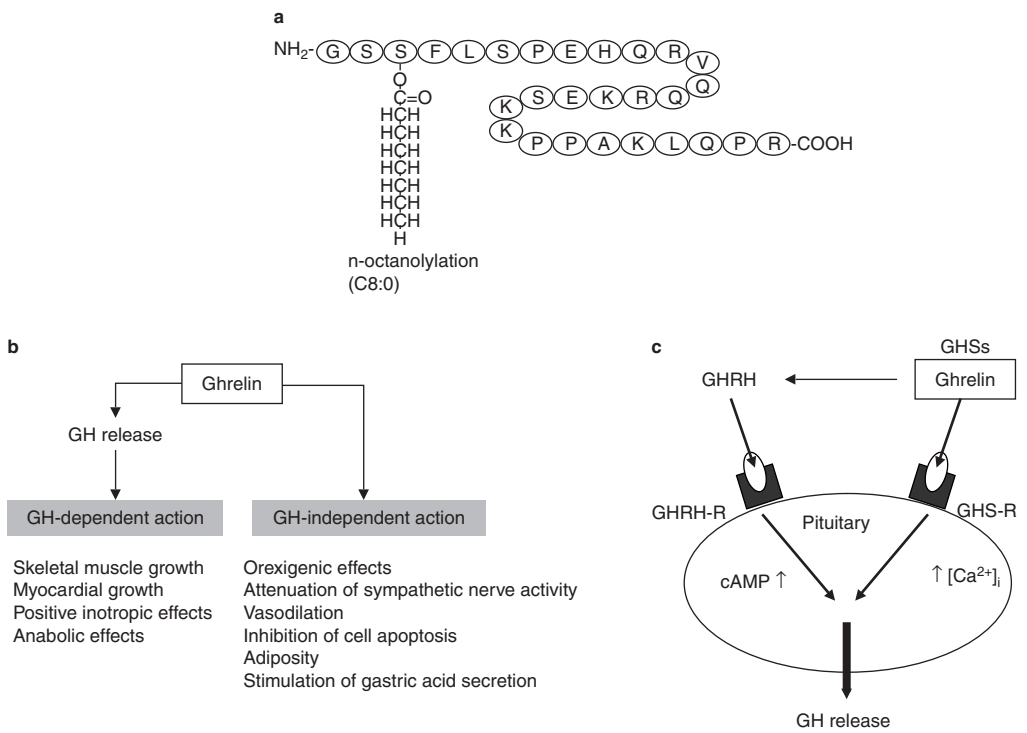

Fig. 1. (**a**) Structure of human ghrelin. Human ghrelin is a 28-amino-acid peptide containing an n-octanoyl modification. (**b**) Biological actions of ghrelin: growth hormone (GH)-dependent and -independent mechanisms. (**c**) Stimulation of GH release by GH-releasing hormone (GHRH) and ghrelin. GHRH acts on the GHRH receptor (GHRH-R) through a cyclic adenosine monophosphate (cAMP)-dependent mechanism, whereas ghrelin and GH secretagogues (GHS) bind to the GHS receptor (GHS-R), followed by the release of Ca^{2+} from intracellular stores. Ghrelin also stimulates GHRH production. ↑ indicates increase.

and function are well established.[13] Patients with GH deficiency have abnormalities of cardiac structure and function, such as reduced cardiac mass and impaired diastolic filling. GH supplementation has beneficial effects on myocardial structure and function in some patients with CHF.[14-16] Thus, GH and IGF-1 play a role as anabolic hormones in the regulation of cardiac development and performance. Considering the haemodynamic and anabolic effects of GH/IGF-1, ghrelin may have beneficial effects on left ventricular (LV) function and energy metabolism in CHF through GH-dependent mechanisms. On the other hand, ghrelin may have direct cardiovascular and metabolic effects through GH-independent mechanisms: (i) GHS-R messenger RNA (mRNA) is detected not only in the hypothalamus and pituitary, but also in the heart and blood vessels;[17] (ii) stimulation of the GHS-R has been shown to prevent cardiac damage after ischaemia-reperfusion in hypophysectomised rats;[18] (iii) ghrelin inhibits apoptosis of cardiomyocytes and endothelial cells *in vitro*;[19] (iv) intravenous injection of ghrelin decreases arterial pressure and increases cardiac output in healthy humans;[17] and (v) ghrelin acts directly on the CNS to decrease sympathetic nerve activity.[20,21] These findings raise the possibility that administration of ghrelin may have beneficial haemodynamic effects in patients with CHF.

In patients with end-stage CHF, LV dysfunction as well as cardiac cachexia are observed.[22-24] Cardiac cachexia, which is a catabolic state characterised by weight loss and muscle wasting, is associated with hormonal changes and cytokine activation in patients with CHF.[23-29] Importantly, the presence of cardiac cachexia is a strong independent risk factor for mortality in patients with CHF.[30] Thus, cardiac

cachexia and LV dysfunction are both therapeutic targets in the treatment of CHF.

Interestingly, peripheral and intracerebroventricular administration of ghrelin have been shown to stimulate food intake and increase bodyweight through GH-independent mechanisms.[31] In addition, ghrelin decreases fat utilisation and increases carbohydrate utilisation through a GH-independent mechanism.[32] Taking these results together with the GH-dependent anabolic effects of ghrelin, this peptide may cause a positive energy balance in CHF through GH-dependent and -independent mechanisms. Thus, ghrelin may be used as an anti-cachectic drug. This article summarises the therapeutic potential of ghrelin in the treatment of CHF.

1. Growth Hormone (GH) Secretagogues and the Discovery of Ghrelin

In addition to the physiological stimulation by GHRH, release of GH from the pituitary is stimulated by small synthetic molecules called GHS.[33-35] They act through the GHS-R, a G-protein-coupled receptor,[36] for which the ligand was unknown until the discovery of ghrelin. GHS are synthetic peptidyl and non-peptidyl molecules that have strong, dose-dependent GH-releasing activities *in vivo*. GHS are a heterogenous group but they have the following common characteristics: GHS are synthetic substances, they stimulate GH release from the pituitary, and they act through the GHS-R but not the GHRH receptor. The GHS family includes peptidyl molecules such as SKF 110679 (GH releasing peptide-6) and examorelin (hexarelin), and non-peptidyl molecules such as L 692429 and ibutamoren (MK 0677). In 1995, Merck & Co. discovered ibutamoren, which has good bioavailability and long-lasting effects after oral administration.[37] These GHS compounds have entered clinical trials for therapeutic indications that include idiopathic GH deficiency states, stimulation of anabolic processes in the elderly and supportive therapy in catabolic wasting conditions.

GHRH acts on the GHRH receptor through a cyclic adenosine monophosphate (cAMP)-dependent mechanism (figure 1c). On the other hand, GHS 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, Kojima et al.[10] found that the GHS-R was activated by stomach extracts. Thus, ghrelin, an endogenous ligand specific for the GHS-R, was successfully isolated from the human and rat stomach in December 1999.[10] Human ghrelin is homologous to rat ghrelin apart from two amino acids.

2. Production and Distribution of Ghrelin and its Receptor

Ghrelin is produced mainly in the stomach. To date, four types of endocrine cells (the enterochromaffin-like cells, the D cells, the enterochromaffin cells and X/A-like cells have been identified in the oxyntic mucosa of the stomach.[38] Date et al.[39] have reported that the X/A-like cells) whose hormonal product had not previously been clarified, secrete ghrelin. Ghrelin is not secreted into the gastrointestinal tract but is rather secreted into the blood vessels. Thus, the plasma ghrelin level is relatively high (100–120 fmol/mL).[40] The plasma ghrelin level falls markedly following gastrectomy.[41,42] Ghrelin is also produced in the small and large intestines and is detected in a limited region of the hypothalamic arcuate nucleus that is involved in the regulation of food intake.[10] 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 a cloning strategy.[36] The GHS-R is a G-protein-coupled receptor with seven transmembrane domains that is present in a variety of tissues including the pituitary and hypothalamus, and is distinct from the GHRH receptor. Interestingly, the GHS-R is detected in the cardiac ventricles and blood vessels, suggesting that ghrelin may cause 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 minutes after a bolus of ghrelin, and the elevation of the GH level lasted for longer than 60 minutes.[17]

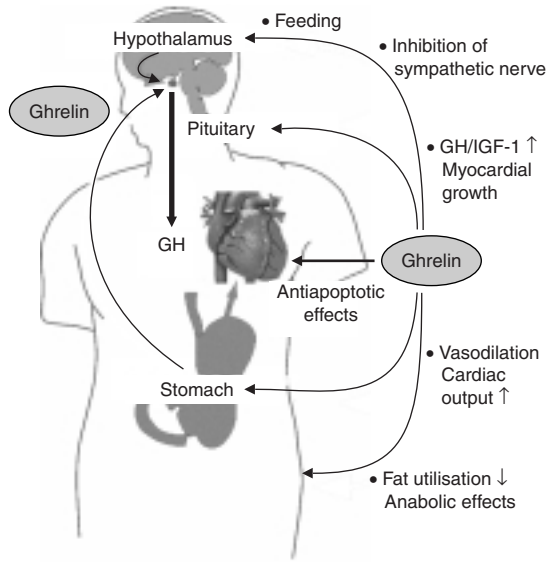

Fig. 2. A schematic illustration of various biological functions of ghrelin. **GH** = growth hormone; **IGF-1** = insulin-like growth factor-1; ↑ indicates increase; ↓ indicates decrease.

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.

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,[14-16] 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.

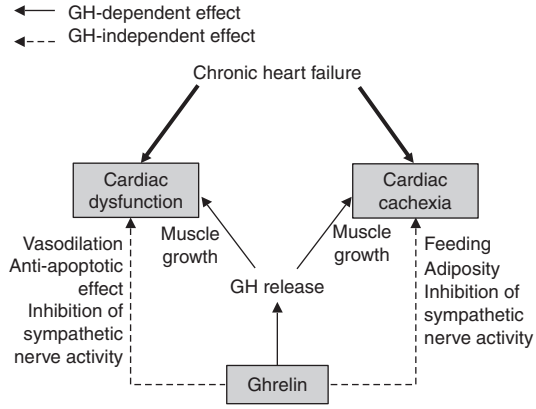

Fig. 3. Characteristics of end-stage heart failure and therapeutic effects of ghrelin. Left ventricular (LV) dysfunction and cardiac cachexia are often observed in patients with severe heart failure. Ghrelin improves LV dysfunction and attenuates the development of cardiac cachexia through a growth hormone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