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Heal the heart through gut (hormone) ghrelin: a potential player to combat heart failure

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Abstract

Ghrelin, a small peptide hormone (28 aa), secreted mainly by X/A-like cells of gastric mucosa, is also locally produced in cardiomyocytes. Being an orexigenic factor (appetite stimulant), it promotes release of growth hormone (GH) and exerts diverse physiological functions, viz. regulation of energy balance, glucose, and/or fat metabolism for body weight maintenance. Interestingly, administration of exogenous ghrelin significantly improves cardiac functions in CVD patients as well as experimental animal models of heart failure. Ghrelin ameliorates pathophysiological condition of the heart in myocardial infarction, cardiac hypertrophy, fibrosis, cachexia, and ischemia reperfusion injury. This peptide also exerts significant impact at the level of vasculature leading to lowering high blood pressure and reversal of endothelial dysfunction and atherosclerosis. However, the molecular mechanism of actions elucidating the healing effects of ghrelin on the cardiovascular system is still a matter of conjecture. Some experimental data indicate its beneficial effects via complex cellular cross talks between autonomic nervous system and cardiovascular cells, some other suggest more direct receptor–mediated molecular actions via autophagy or ionotropic regulation and interfering with apoptotic and inflammatory pathways of cardiomyocytes and vascular endothelial cells. Here, in this review, we summarise available recent data to encourage more research to find the missing links of unknown ghrelin receptor–mediated pathways as we see ghrelin as a future novel therapy in cardiovascular protection.

Keywords Ghrelin · GHSR-1A · Heart · Blood vessels · Cardiomyocytes

Introduction

Ghrelin, a 28 amino acid (aa) peptide, was first purified from rat stomach in 1999 [1]. Although this peptide hormone is produced predominantly in X/A cells or 'ghrelin cells' of gastric oxyntic mucosa of duodenum in adults and ε cells of from pancreatic islets in foetus [2], ghrelin mRNA and protein are also distributed in different tissues including cardiomyocytes and endothelium of blood vessels [3, 4]. In recent years, studies revealed a plethora of functions of ghrelin including appetite stimulation, gut motility, gastric acid secretion, taste sensation and glucose metabolism, sleep/wake rhythm, learning and memory, reward seeking behaviour, and cardiovascular protection [5]. Ghrelin induce GH secretion from pituitary, called growth hormone secretagogue (GHS), and can regulate hypothalamus-pituitary axis [6, 7]. In nomenclature, 'ghre' comes from the Proto-Indo-European root of the word 'grow', and 'relin' is the short form that refers to its GH-'releasing' activities [8]. There are two major forms of ghrelin: biologically active acyl-ghrelin and non-active desacyl ghrelin [9]. It bears N-octanoylation at its third serine residue, which is necessary for active receptor binding to exert biological effects. In healthy individuals, level of non-acylated serum ghrelin is significantly higher compared with the bioactive acylated ghrelin [10], but this balance is disrupted in pathological conditions [11].

From the middle of the twentieth century, the heart has also emerged as endocrine organ that maintain homeostasis in the cardiovascular system and also other organs that control its function [12]. The term 'cardiokine' is used to describe proteins/peptides secreted from the heart, and particularly, 'cardiomyokines' which are secreted from cardiomyocytes [13]. Cardiac cells bear GHS receptors, which indicate ghrelin-mediated biological actions within the heart [14]. Basically, this gastric hormone establishes a crucial endocrine link with the heart [15]. Ghrelin-mediated cardiovascular

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protective effects have been reported in both GH-dependent and GH-independent manner [16–18].

In this review article, we will incript the most recent cardiovascular protective evidences of ghrelin and the background mechanisms involved to highlighting its possible future therapeutics potentialities.

Distribution, chemical structure, and synthesis of ghrelin

Ghrelin is not exclusively but predominantly produced by the gastric cells and also expressed in several different organs, including the pancreas, kidney, testes, ovary, pituitary gland, hypothalamus, intestine, lymphocytes, and/or placenta [3]. Ghrelin has a highly conserved sequence in almost all vertebrates [19]. The ghrelin gene of human is located on the 3p25–26 chromosome and consists of three introns and five exons which encode a transcript of 511 bp, corresponding to 117 amino acid long precursor named preproghrelin [20]. The precursor of the hormone, proghrelin, yields two mature peptides, ghrelin (28 aa) and obestatin (23 aa). Preproghreln enters the endoplasmic reticulum, and signal peptide sequence of 23 aa is removed by the enzyme prohormone convertase 1/3 (PC 1/3) to generate ghrelin [21]. The same enzyme perhaps plays a critical role in processing of obestatin. A portion of ghrelin pool, during maturation, undergoes post-translational acylation, commonly, octanoylation of the serine at the 3rd position by the enzyme ghrelin O-acyltransferase (GOAT) [6, 22] (Fig. 1a). Other portion of ghrelin is called unacyl-ghrelin, but inactive as it cannot bind to its receptor GHSR and exerts downward molecular actions [23]. The presence of n-octanoyl group modification at the side chain of Ser3 in the peptide determines the potency of active ghrelin to bind with its receptor to be active form of ghrelin [24].

Ghrelin hormone receptors in the cardiovascular system

Ghrelin receptor (GHSR) was first isolated from the pituitary gland [25], and later, it was found in different parts of brain and peripheral organs like the heart and pancreas GI tract [5] [26]. The human GHSR gene is located on chromosome 3q26.2 and mostly conserved across species [27] and encodes two splicing variant, GHSR-1A and GHSR-1B/non-GHSR-1A [25, 28]. Interestingly, an additional subtype of ghrelin receptor, different from classical GHSR-1A and 1B, has been identified in H9C2 cardiomyocytes and endothelial cell. Interestingly, both acylated and non-acylated ghrelin can bind to GHSR-1B subtype [29]. GHSR-1A and GHSR-1B are 7 and 5 spanned trans-membrane G protein-coupled receptor, respectively. Although functions of GHSR-1A are well documented, that of GHSR-1B are still matter of conjecture [25, 30, 31] (Fig. 1b). A minimal sequence is needed to activate GHSR-1A, which is a short N-terminus tetra- or pentapeptides including the first *Gly-Ser-Ser[n-octanoyl]-Phe* amino acids that constitute the 'active core' of ligand to bind stringentically with its receptor and activate GHSR-1A-mediated cascades [32]. GHSR-1A is an important player for orexigenic effects of ghrelin but non-type GHSR-1A may exert other physiological functions of ghrelin [33]. In addition, a type B scavenger receptor CD36 (84-kD glycoprotein) is found in rat and human cardiovascular tissues [34–36] and can bind with synthetic peptidyl GHSs, such as [^{125}I] Tyr-Ala-hexarelin. However, potential connection between CD36 receptor and endogenous ghrelin down cascades is still a matter of conjecture and requires further studies. Using a [^{125}I] His9-ghrelin-binding assay, the pervasiveness of GHS receptors in the cardiovascular system has been confirmed in the human saphenous veins, coronary artery, left ventricle, and right atrium [37].

Different subtypes of ghrelin receptors vary greatly in structure as well as in their biological effects on the heart and blood vessels. Elucidation of these receptors in the heart and better understanding of binding sites for ghrelin might lead to the development of new synthetic agents with all the beneficial effects on the heart cells described further in this article without the concomitant effects on GH release.

Cardiovascular protection by ghrelin

Endogenous and exogenous ghrelin is active in the heart as well as in the blood vessels [38–42]. Chronic ghrelin administration heals left ventricular dysfunction and cardiac cachexia significantly by vasodilatation in rat model [43] and prevents cardiac hypertrophy, fibrosis after MI, and ischemia/reperfusion injury resulting in reduced mortality rate in in vivo and isolated murine heart disease model [44–46]. Ghrelin treatment also reduced arterial blood pressure, improved ventricular function and endothelial dysfunction, and significantly increased cardiac capacity and oxygen consumption during exercise in patients with chronic heart failure (CHF) [47]. Several beneficial effects of ghrelin on the cardiovascular system are summarised in Fig. 2.

Ghrelin ameliorates myocardial infarction

Myocardial infarction (MI) is a term used when a patch of cells die in myocardium due to reduced blood flow to heart resulting in lack of oxygen supply. This occurs due to formation of plaques in interior walls of arteries [48]. Multiple evidences indicate role of ghrelin-mediated cardioprotection in post-MI rats by turning on pro-survival mechanisms [49]. Acyl ghrelin treatment for 2 weeks after MI reduces

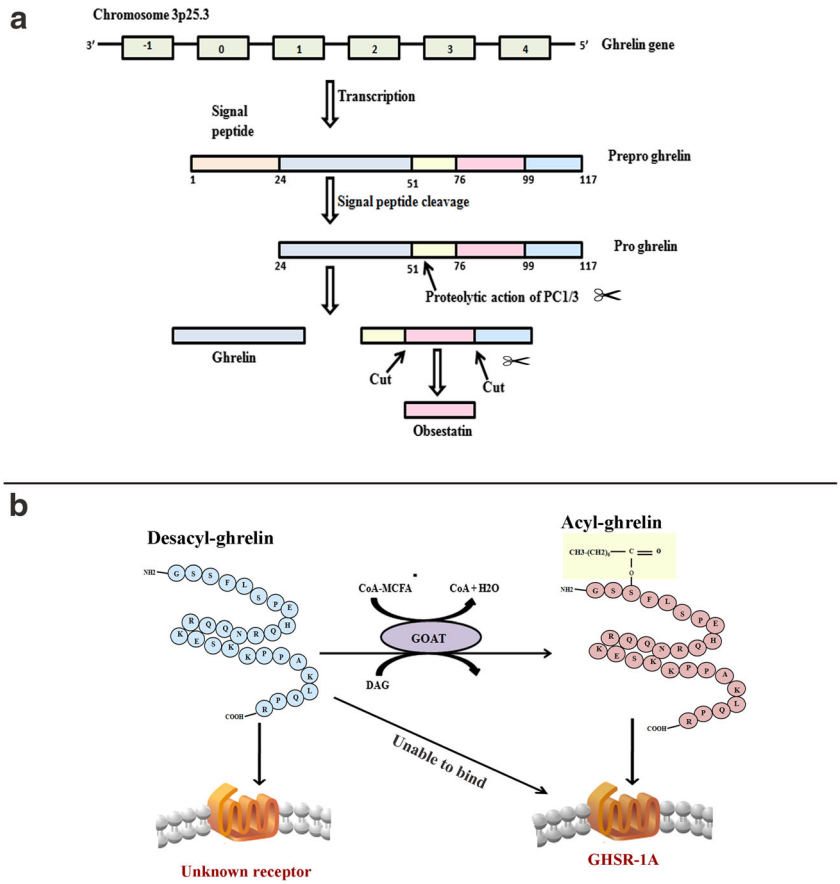

Fig. 1 Human ghrelin gene and its products. **a** Post-translational processing of the human ghrelin gene. mRNA is transcribed to preproghrelin, further processed proghrelin precursor by prohormone convertase 1/3 (PC 1/3). Proghrelin is cleaved at arginine-28 of proghrelin, forming mature 28 amino acid long ghrelin peptide. The majority of the peptide remains unacylated (des-acyl ghrelin) and a smaller portion of it undergoes a post-translational modification. Esterification of a fatty acid on Ser3 by the enzyme GOAT has been done to generate acyl ghrelin. The 23 amino acid peptide obestatin is also generated from the proghrelin precursor. **b** Acylated ghrelin can bind to GHSR-1A, a 7 spanned GPCR but desacyl ghrelin is unable to bind to GHSR-1A and act via some unknown receptors

cardiomyocyte mortality and normalises heart rate by mitigating sympathetic activation and left ventricular function in murine models in vivo [44, 50]. Short-term exposure is effective too, even in less than 2-h exposure after MI. The cell mortality rate has attenuated in rat [51, 52]. In chronic MI patients, level of plasma ghrelin has been reported to be significantly low [53–55]. Increased mortality in post-MI condition was also evident in ghrelin-KO mice which were efficiently reverted by exogenous ghrelin treatment [55, 56]. Chronic administration of ghrelin very significantly suppressed the MI-induced increase in heart rate and plasma norepinephrine conc. to levels at par with sham-operated controls [44, 57]. Plasma ghrelin [58] or locally produced myocardial ghrelin was found to be upregulated in isoproterenol-induced myocardial injury and fibrosis as well as ischemic heart disease, acting via non-GHSR-1A receptors [58, 59].

Ghrelin was reported to reduce the post-MI scar, ameliorate inflammatory cytokines such as IL-1β and TNF-α, and regulate activinA/follistatin imbalance in rat models in vivo [60, 61]. Wang et al. showed ghrelin-mediated amelioration of Ang II-induced myocardial fibrosis by upregulating PPAR- , and PPAR- antagonist GW9662 treatment counteracted the effects [62]. In another study by Eid et al. [63], 'Attenuation of SOCS3' and 'induction of JAK2/STAT3' were identified as pathways by which ghrelin acts in post-MI remodelling. Anti-apoptotic function of active ghrelin was also observed via activation of Raf-MEK1/2-ERK1/2 or inhibiting oxidative stress and inflammation via TLR4/NLRP3 signalling in experimental MI animal models [64, 65]. In post-MI rat's aorta, vasodilation and oxidative stress reduction by acylghrelin were found to be mediated by inhibiting ACE-induced activation of NADPH-dependent oxidase and/or upregulation of eNOS activity [66].

However, more in-depth investigations are required to unravel therapeutic effects of ghrelin in early myocardial infarction.

Ghrelin heals cardiac hypertrophy and fibrosis

Cardiac hypertrophy is one of the adaptive responses of the heart in chronic hemodynamic overload and/or persistant MI that increases the risk of heart failure [67]. Inflammatory cytokines play pivotal roles in the progression of cardiac hypertrophy [68, 69]. Ghrelin attenuates those inflammatory cytokines [70] and thus can be beneficial in cardiac hypertrophy. Ghrelin can induce parasympathetic cardiac-vagal nerve

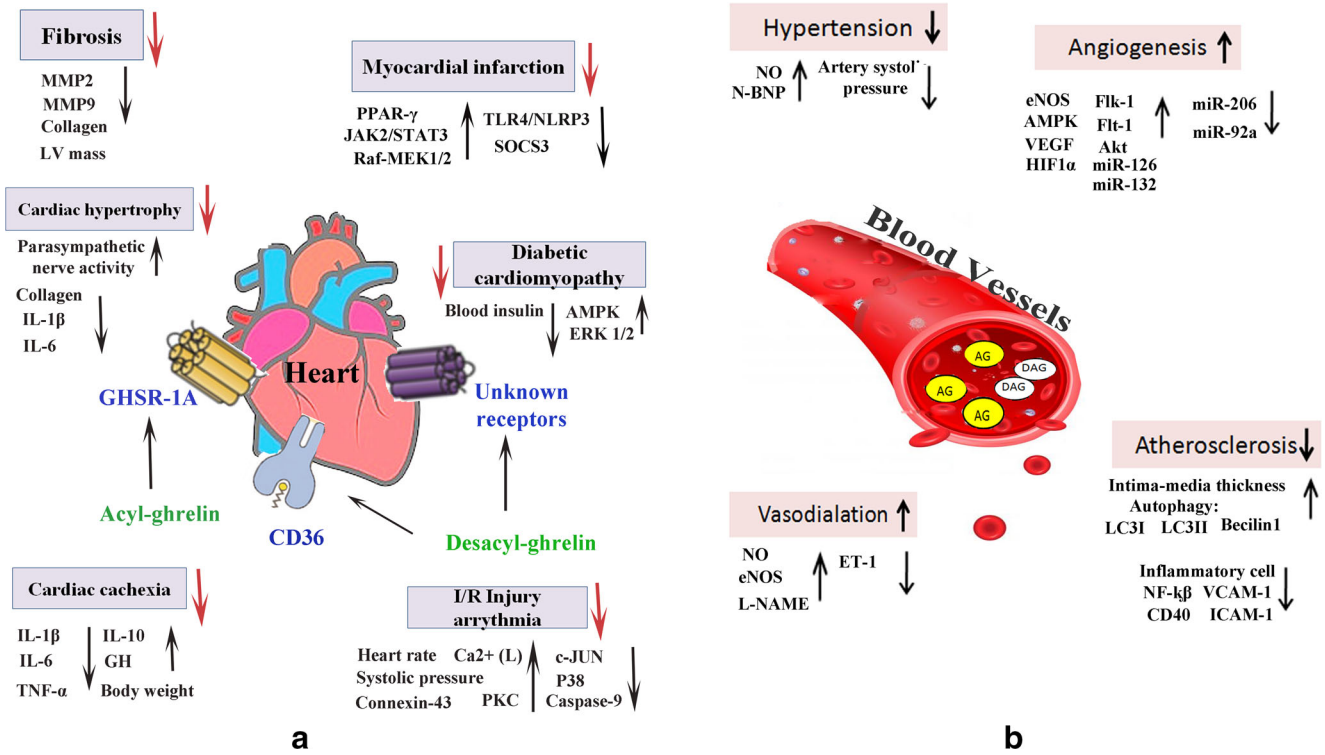

Fig. 2 Summary of role of ghrelin as cardioprotective peptide. **a** Schematic diagram showing ghrelin and desacyl ghrelin–mediated cardiac protection in different diseases like myocardial infarction, hypertrophy, fibrosis, cardiac cachexia, diabetic cardiomyopathy, arrythmia, and I/R injury. **b** Actions of ghrelin on vasculature showing healing properties in hypertension and coronary artery disease and atherosclerosis and promoting angiogenesis and vasodilatation. Upward arrow signifies upregulation and downward arrow signifies downregulation of particular molecule or pathway depicted in the figure. MMP, matrix metalloproteinases; IL-2, interleukin-2; TNF-α, tumour necrosis factor-α; GH, growth hormone, PPAR-γ, peroxisome proliferator-activated receptors-γ; JAK-2, janus kinase-2; STAT-3, signal transducer and activator of transcription-3; TLR4, Toll-like receptors-4; NLRP3, NLR family pyrin domain containing 3; AMPK, AMP-activated protein kinase; ERK1/2, extracellular signal-regulated kinases 1/2, PKC, protein kinase-C; Ca^{2+}, calcium ions; NO, nitric oxide; N-BNP, N terminal-proB-type natriuretic peptide; eNOS, endothelial nitric oxide synthetase; ET-1, endothelin-1; Flk-1, VEGF receptor 2; Flt-1, VEGF receptor 1; VEGF, vascular endothelial growth factor; Akt, protein kinase B; miR, microRNA, L-NAME, N(δ)-nitro-L-arginine methyl ester; LC3 I, microtubule-associated protein 1A/1B-light chain 3; CD40, cluster of differentiation-40; NFκ-β, nuclear factor kappa-light-chain-enhancer of activated B cells

activity [71] which was attenuated in ventricular hypertrophic condition [72]. Ghrelin-KO mice demonstrated worse pathophysiological condition by attenuating cholinergic anti-inflammatory pathway and parasympathetic nerve activity but inducing pro-inflammatory IL-1β and IL-6 levels, which were reversed significantly by exogenous ghrelin [46, 73].

The excess deposition of collagen in the heart ECM by several stimuli is a common pathophysiological feature of cardiac hypertrophy or cardiac remodelling after heart injury [74]. Both acylated and unacylated ghrelin inhibited cardiomyocyte death, and production of excess ECM proteins in vivo in doxorubicin-induced cardiomyopathy [75, 76] was observed. Significant anti-fibrotic effects of ghrelin have been also demonstrated in other experimental models of cardiac injury, such as isoproterenol administration, myocardial infarction (MI), and spontaneous or diabetes-associated hypertension [60, 77–80]. Exogenous -adrenergic agonist isoproterenol induces heart injury that also leads to fibrosis and increases myocardial ghrelin expression and plasmatic acylated ghrelin levels [77, 78], although the mechanisms of such protective measure are still unclear. Desacyl ghrelin also displays anti-fibrotic actions by GHSR-independent pathways [78] and significantly blunts the induction of MMP-2 and MMP-9 that could be inferred as inhibition of overall fibroblast activity [60]. The synthetic GH-secretagogue, hexarelin, also prevents cardiac fibrosis by inducing MMP-2 and MMP-9 activity in spontaneously hypertensive rats [79]. However, desacyl ghrelin was shown to have no effect on other MMPs like MMP-8 and MMP-13 which are also fibrotic mediators [80]. In db/db mice, unacylated ghrelin impaired collagen accumulation by upregulating adiponectin expression [80], which is a well-known regulator of cardiac hypertrophy and fibrosis [81, 82].

Ghrelin and cardiac cachexia

Cardiac cachexia (CC), severe weight loss due to heart disease, disrupts catabolic and anabolic balance of the body [83],

promotes hormonal changes and cytokine activation [84, 85], and in turn, provokes systemic tissue wasting, skeletal muscle loss, and bone loss too [86–89]. Ghrelin secretion has been proved to counter energy deficit against starvation-related cachexia [90] and control adaptive feeding response [91]. Being a strong adipogenic and orexigenic molecule, it induces weight gain and adiposity [92] by increasing not only in body weight and body fat mass but also in lean tissue mass and reduction of myostatin plasma levels [93]. The resistance of heart failure (HF) patients to the effects of appetite-stimulating ghrelin was put forward as one of the possible contributing factors in the development of cardiac cachexia [94]. Patients with HF and CC have significantly higher plasma ghrelin levels than in those without CC and healthy subjects, which indicates a compensatory or counteracting mechanism condition of anabolic/catabolic imbalance in CC [95]. Exogenous ghrelin found to be beneficial in 2 ways as it increases cardiac output and decreases systemic vascular resistance in patients with cardiac cachexia [96, 97]. In addition, exogenous administration of ghrelin has been observed to elicit a potent, long-lasting stimulation of food intake via induction of neuropeptide Y (NPY) neurones in the hypothalamic arcuate nucleus [98–100]. Ghrelin analogues BIM-28131 and BIM-28125 improve body weight [43], increasing appetite in experimental heart failure model by regulating MuRF-1 and MAFbx [101] as well as myostatin [102] expression.

These results raised the possibility that increased plasma ghrelin may play a compensatory physiological role when balance of anabolic/catabolic factors is disrupted in cachectic patients with chronic HF.

Ghrelin and diabetic cardiomyopathy

It is well established that individuals with diabetes have a significantly higher risk of developing cardiovascular diseases including cardiomyopathy (CM) and heart failure [103, 104]. Chronic heart failure is the reason for ~70% of diabetes-related deaths [105]. Diabetes can directly cause myocardial injury, resulting in a distinct disease called 'diabetic cardiomyopathy (CM)'. Data from epidemiologic, experimental, and clinical studies have shown diabetes-related structural and functional cardiac changes including higher left ventricle (LV) mass, wall thickness, and/or arterial stiffness [106] caused by deposition of advanced glycation end-products (AGEs) and collagens [107, 108]. The cardioprotective effects of hexarelin and ghrelin have been well investigated in diabetic animal models, and its beneficial roles are evident in several studies [109–113].

Firstly, the acylated ghrelin increases blood glucose concentrations and decreases insulin in rodent model [114, 115], which are not favourable for diabetic individuals. In contrast, desacyl ghrelin decreases glucose release and improves insulin sensitivity [116, 117], stimulating lipid accumulation [118] in adipocytes. Preliminarily, desacyl ghrelin has been demonstrated to have beneficial effects on cardiovascular system and metabolism of glucose and lipid [119, 120]. Obestatin, another product of pro-ghrelin, effectively protected STZ-induced myocardial dysfunction [121]. A negative correlation between plasma ghrelin was documented in hypertensive population [122] and obese patients [123]. Exogenous ghrelin administration to overweight patients strongly improves their insulin sensitivity [124] and that protects the heart by inhibiting excessive collagen deposition in ECM and inducing autophagic signalling via the pro-survival AMPK/ERK1/2 signalling pathways in db/db mice [80]. Desacylated form is unable to stimulate release of growth hormone (GH) [28] and indicates that its role in diabetic cardiomyopathy protection is via non-GHSR-1A receptors. The therapeutic value of desacyl ghrelin in diabetes management would be potentially high. Desacyl ghrelin treatment alleviated diabetic cardiomyopathy by improving the contractile function of left ventricle, reducing cardiac fibrosis, and activating cardiac autophagy [80].

Ghrelin-mediated attenuation of hypertension

Hypertension can be defined as a chronic elevation of systematic arterial pressure above a certain threshold value that causes a cardiovascular risk [125]. In a large middle-aged hypertensive cohort study, significant negative association between ghrelin and blood pressure has been observed [126]. Furthermore, ghrelin gene Arg51Gln mutation was proved to be an independent risk factor of hypertension [127]. In contrast, plasma ghrelin levels are significantly increased in idiopathic pulmonary hypertension (PH) and are positively correlated with NO levels, levels of N-BNP, pulmonary artery systolic pressure, and RV diameter [128]. Microinjection of ghrelin into the nucleus of the solitary tract of rats significantly decreased the mean arterial pressure and heart rate, but in contrast, unilateral microinjection of ghrelin into the area postrema, rostral, and caudal ventrolateral medulla caused no significant changes in the mean arterial pressure and heart rate [129]. In pulmonary hypertension rat models, it significantly attenuated hypertension, pulmonary vascular remodelling, right ventricular hypertrophy, right ventricular diastolic disturbances, wall thickening of peripheral pulmonary arteries, and also, ameliorated left ventricular dysfunction [130].

This correlation between ghrelin and hypertension is muddled when these parameters were assessed during pregnancy. In normotensive pregnant women, ghrelin was negatively correlated with blood pressure, whereas in hypertensive non-pregnant women, there was a significant positive correlation [131]. In spontaneously hypertensive pregnant rats, plasma ghrelin levels were significantly higher compared with controls [132]. But ghrelin level in the stomach is unchanged, and the level of ghrelin mRNA in the placenta was lower in the hypertensive animals, indicating possibly a different local

production of ghrelin during pregnancy is regulated differently [132].

Ischemia-induced injury and arrhythmias control by ghrelin

Ischemia/reperfusion (IR) injury may be defined as damage of cells that results from a period of ischemia followed by re-establishment of blood supply at the ischemic site [133]. It is a significant cause of myocardial morbidity after an acute MI [134–136]. Ghrelin has been shown to be beneficial against I/R injury in numerous organs including the liver, kidneys, intestines, spinal cord, and pancreas in animal models [137–141]. In the heart, both ghrelin and its analogue hexarelin have been shown to exert cardioprotective effects after I/R injury [110, 111]. Increased coronary flow, heart rate, and LV systolic pressure, as well as reduced LV end-diastolic pressure and myocardial release of lactate dehydrogenase and myoglobin, have been observed after administration of ghrelin during I/R injury in isolated rat hearts [45]. Preservation of electrophysiological properties by ghrelin by regulating both voltage-gated L-type calcium and sodium currents has also been reported in isolated I/R injury heart model [142]. The mechanism of protection provided by GHSs after I/R injury is GH independent, which results in an increased maximum binding capacity of ghrelin on sarcolemmal membranes [45]. Endoplasmic reticulum stress inhibition and blocking of pro-apoptotic processes through a Ca^{2+}/calmodulin/AMPK pathway are one of the major mechanisms of actions of ghrelin [143]. GHSs were also shown to prevent the phosphorylation of pro-apoptotic proteins p38, c-Jun NH (2)-terminal kinase, and caspase-9 [142]. Other potential pathways including protein kinase C or recovering sarcolemmal Ca^{2+} are induced [109, 111].

In ghrelin KO mice, malignant arrhythmias worsen that can be reversed through exogenous ghrelin replacement [50]. Ghrelin can significantly decrease vulnerability of ventricular arrhythmias in rat MI model, by increasing expression of connexin-43 in the myocardium [144, 145]. Intravenous administration after ligation of the left coronary artery ameliorates ventricular tachyarrhythmia preventing the loss of phosphorylated connexin-43 through higher parasympathetic activity of vagal nerve [145] and adrenergic response [50, 56, 146].

Vasodilatation and hemodynamic effects of ghrelin

Endothelial cells are very important constituents of blood vessels which play critical role in cardiovascular homeostasis [147]. Immuno-reactive ghrelin protein has been identified in endothelial cells of the human artery and vein [40] that suggests this peptide may be involved in endothelial function in vascular system. Low level of ghrelin is directly associated with high blood pressure [126]. Serum ghrelin level was found to be significantly lesser in obese patients having metabolic syndromes with impaired endothelial function [123, 148]. Intravenous injection of acyl-ghrelin (10 μg/kg body weight dose) to 6 healthy volunteers in randomised fashion leads to beneficial hemodynamic alterations and increased cardiac output without any heart rate alteration [149].

There are several hypotheses of ghrelin-mediated vasodilation that have been proposed so far. Vascular homeostasis is maintained by balance of endothelium-derived relaxing factor nitric oxide (NO) and contracting factors, endothelin (ET)-1 [150]. Intravenous ghrelin replacement reduces blood pressure in human as well as rabbit without any change in heart rate [149, 151]. Further, Okomura et al. [152] explained this event is a GH/insulin-like growth factor (IGF)-I/nitric oxide (NO)-independent mechanism. Interestingly, ghrelin (0.1-300 nM) has been demonstrated as a good vasodilator in denuded mammary gland artery, which indicates another endothelial cell-independent mechanism of vasodilatation [153].

In contrary, ghrelin exposure stimulates NO production in human and bovine aortic endothelial cells in vitro in a time- and dose-dependent manner that can be blocked by GHSR-1A receptor blocker [d-Lys3]-GHRP-6 [154]. Chronic ghrelin treatment in isolated aortic rings of GH-deficient dwarf rats significantly increased endothelium-dependent relaxation that has been reversed by inhibiting L-NAME as compared with those given placebo. This effect further demonstrated that treatment with ghrelin increased aortic endothelial NOS expression, and this peptide-mediated vasodilation can be reversed by selective eNOS inhibitors [155]. Ghrelin decreases sympathetic nervous outflow upon stimulation of the ghrelin receptor in neuronal cells of the nucleus of the solitary tract, and microinjection of ghrelin at a very low dose in this area was sufficient to induce an average drop in mean blood pressure [126]. Intracerebroventricular injection (ICV) of ghrelin (1–5 nM) decreased mean arterial pressure in rabbits [151].

Taken together, these results suggest that ghrelin binds to GHSR-1A and can act as vasodilator via both NO-dependent and NO-independent pathways.

Ghrelin and coronary artery disease, atherosclerosis

Several studies support the notion that high ghrelin is linked to reduced early atherosclerosis [156–159]. However, relationship between ghrelin concentration and intima-media thickness (IMT) in coronary atherosclerosis human patients showed conflicting results [156–158, 160, 161]. The positive association between carotid IMT and plasma ghrelin levels was also reported [160, 161]. In a long-time follow-up study spanning 19 years involving over 1000 individuals from Finland, a high plasma ghrelin level was shown to protect against coronary heart disease [162].

Ghrelin to GHSR-1A binding was found to be upregulated up to 3- to 4-fold in coronary arteries and saphenous vein grafts of atherosclerotic rat model [163]. Ghrelin controls atherosclerotic pathogenesis, attenuating inflammation, endothelial dysfunction, and endoplasmic reticulum stress [164], and increases NO bioactivity and eNOS levels [123, 155]. In addition, ghrelin reduces inflammatory cell activation on the endothelial by NF-κB, CD40, vascular cell adhesion molecule 1 (VCAM-1), and intracellular adhesion molecule-1 (ICAM-1) in mice [165–168]. Moreover, ghrelin receptor deficiency has been found to aggravate atherosclerotic plaque instability and worsen vascular inflammation, suggesting a possible protective mechanism for ghrelin against atherosclerosis and its complications.

In contrast, different age and grade of atherosclerosis in the patients of these studies may explain discordant findings. In renal transplant patients, elderly hypertensive patients and patients with metabolic syndrome, both acyl-ghrelin and des-acyl ghrelin were found to be negatively correlated with IMT [157–169]. Reduced plasma ghrelin levels were closely related to angiographically detect complex lesion morphology in patients with coronary artery disease [159]. Another study revealed high plasma ghrelin concentrations to be significantly correlated with increased carotid artery IMT in middle-aged (40–60 years) males [160]. These contradictory findings muddled the hypothesis and led to questions about whether the positive changes by ghrelin in atherosclerosis are multidimensional and case based.

Ghrelin promotes angiogenesis

Angiogenic properties of ghrelin in ischemic tissue are well established in multiple studies [151, 170–173]. Ghrelin significantly induce vascular endothelial growth factor (VEGF) expression in the peri-infarct zone compared with the control group [171]. This pro-angiogenic property was regulated by GHSR1A-mediated AMPK/eNOS pathway and upregulating of HIF1α, VEGF, and its receptors Flk-1, Flt-1 expressions [172]. Knockdown of GHSR-1A by siRNA markedly decreased VEGF along with Akt and AMPK mRNA expression. In conclusion, GHSR-1A gene therapy improves cardiac remodelling and function in rats after MI. This may be a new anti-remodelling target in MI patients [15]. Activation of pro-angiogenic and anti-fibrotic microRNAs (miRs) are key cellular pathways underpinning the protective effect of ghrelin. miR-126, expressed in endothelial cells, accelerates angiogenesis by increasing ERK as well as PI3K/Akt/VEGF signalling pathway, inhibiting Sprouty-related protein (SPRED1) [174, 175]. Enhanced VEGF activity leads to activation of miR-132, ultimately leading to Ras activation, and promotes neovascularisation [176]. Selective inhibition of pro-angiogenic miRs in vitro implicates miR-126 as a possible upstream modulator for miR-132, although future in vivo knockdown studies are needed to confirm this notion [173].

In contrast, several reports have refuted the angiogenic effects of ghrelin in diabetic or diet-induced obese mice [177–179]. miR quantitative analyses have been showed no activation of proangiogenic miR-132 in the saline-treated mice, whereas miR-132 induces the phosphorylation of CREB through inhibition of anti-angiogenic p120RasGTPase-activating protein [180–182]. In addition, ghrelin also inhibited the activation of anti-angiogenic miR-206 and miR-92a. miR-206 demonstrated negative regulation of angiogenesis via direct inhibition of VEGF [183], while miR-92a, another endothelial cell-specific miRNA, negatively regulates integrin, α5, which is essential for the activation of Akt [184]. These contradictory results indicate ghrelin-mediated angiogenesis may be multifactorial in obese mice and depends on other metabolic actions too.

Ghrelin in pathophysiology of chronic heart failure

Chronic heart failure (CHF) is the final outcome of most cardiovascular diseases and major cause of death in CVD patients [185, 186]. Ghrelin has been reported to effectively improve cardiac performance under different pathological conditions of CVD [187, 188]. In CHF-prone patients, short-term intravenous ghrelin infusion increased mean arterial pressure without affecting heart rate [189]. Endogenous ghrelin was attenuated in atria and ventricles of humans with CHF, while GHSR-1A mRNA was increased, possibly because of compensatory mechanism of adaptation [38]. Consistent with other results described before, ghrelin restores cardiac function and appetite in patients with end-stage CHF and cardiac cachexia [190], possibly as beneficial response to the anabolic-catabolic imbalance [95, 191]. Active exogenous ghrelin administration stimulates fatty acid oxidation and inhibits glucose oxidation, thereby revering the altered energy substrate utilization observed with pacing-induced heart failure in animal models [192], and significantly decreases systemic vascular resistance and increases the cardiac output and stroke volume in patients [149, 193]. Chronic ghrelin administration decreased plasma catecholamine levels that regulate sympathetic nervous control of the heart [56], improved ejection fraction in the left ventricle from 27 to 31% very fast, and increased the peak workload, oxygen consumption during exercise in CHF patients [47].

However, these beneficial effects need much larger-scale controlled clinical trials to evaluate role of acyl and des-acyl ghrelin on CHF. If long-term and/or short-term administration of ghrelin proves feasible and significantly effective compared with placebo, this hormone might offer a new miraculous option for the difficult treatment of CHF.

Mechanism of actions

We discussed differential cardioprotective roles of ghrelin and promoted a potential therapeutic role of ghrelin in cardiovascular disease. Now, question is how ghrelin exerts such beneficial effects during efficient types of cardiac-patho-physiology? We shall discuss some of the basic mechanism of actions for evidences of ghrelin-mediated cardioprotection with schematic diagram (Fig. 3).

Autophagy

Cardiomyocytes critically regulate its own protein synthesis, processing, and elimination (proteostasis) for maintaining homeostasis due to their limited ability to divide, and autophagy plays a pivotal role to regulate proteostasis of cardiac cells [194, 195]. Cardioprotection of ghrelin is associated with enhanced autophagy and removal of dysfunctional mitochondria after MI and calcification attenuation in vascular calcification model [59, 194, 196]. Pharmacological stimulation of autophagy with rapamycin (an mTOR inhibitor) during myocardial ischemia protects against post-infarction pathophysiological remodelling [197]. There are several evidences that inhibition of GOAT (ghrelin forming enzyme) attenuates lipotoxicity by restoration of autophagy. This in turn stimulates restoration of AMPK-mTOR or inhibition of nuclear factor-kβ in NAFLD and other disease models [198–200]. Interestingly, IP injection of desacyl ghrelin in obese, db/db T2DM mice protects against diabetic cardiomyopathy by enhancing autophagy via the pro-survival AMPK and ERK1/2 pathways [80]. Similarly, chronic IP administration of exogenous ghrelin improves autophagy in vascular smooth muscle cells from rats with vascular calcification in an AMPK-dependent manner [196]. Nutrient depletion due to fasting, during the ischemic phase activates AMPK, which in turn, inhibits mTOR, the major inhibitor of autophagy in obese mice [201]. Starvation leads to ghrelin over expression [202] and repetitive starvation during hypoxic heart injury, autophagy-lysosome machinery by inducing nuclear translocation of transcription factor EB (TFEB) [197]. During acute cardiac ischemia, mostly, desacyl ghrelin markedly attenuates infarct size, in part, by stimulating autophagy to remove dysfunctional mitochondria after MI and via the pro-survival cellular AMPK/ERK1/2 signalling pathway mice models of diabetic cardiomyopathy [59, 80].

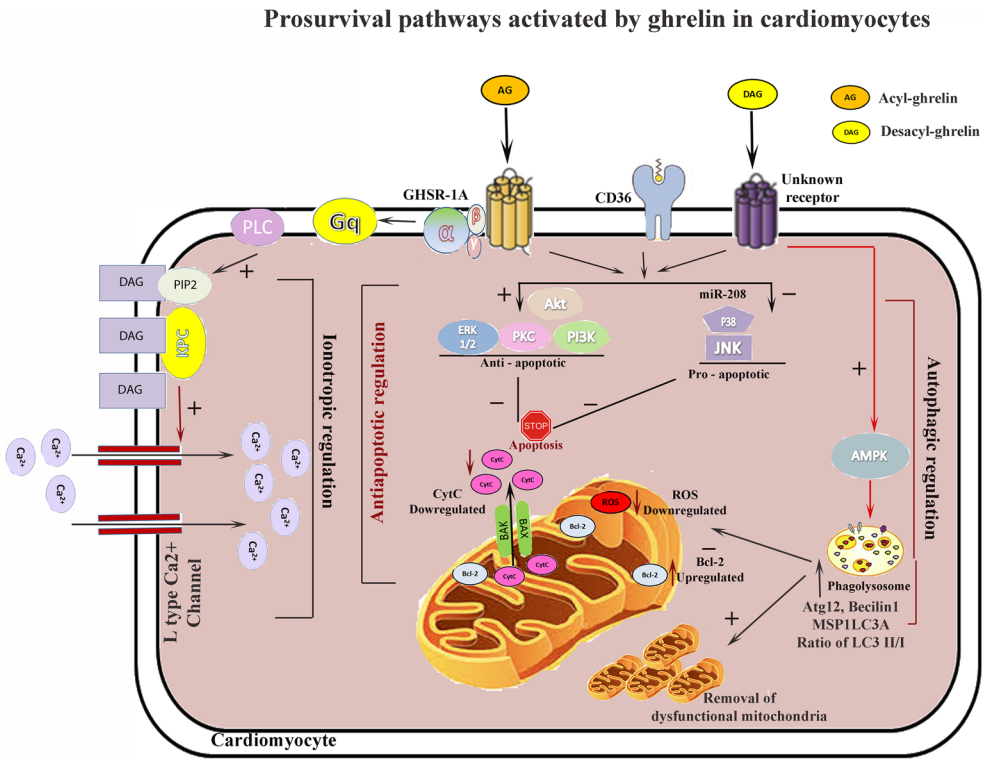

Fig. 3 Mechanisms of actions (MOA) of ghrelin and/or desacyl-ghrelin on a cardiomyocyte. Autophagic, ionotropic, and anti-apoptotic pathways and their regulation are mentioned here. Plus sign (+) signifies upregulation and negative sign (−) signifies downregulation of the particular pathway with arrowheads. AG, acyl-ghrelin; DAG, desacyl-ghrelin(in yellow oval shape); GHSR-1, ghrelin receptor-1; CD36, cluster of differentiation-36; Gq, Gq protein alpha subunit; PLC, phospholipase-C; DAG, diacyl glycerol (in purple box); PIP2, phosphatidylinositol biphosphate; JNK, c-Jun N-terminal kinase; PI3K, phosphoinositide 3-kinases; Cyt-c, cytochrome-C; ROS, reactive oxygen species; Bax, Bcl-2-associated X protein; Bak, BCL2-antagonist/killer; Bcl-2, B cell lymphoma 2 protein; AMPK, 5′ AMP-activated protein kinase; Atg-12, anti-thymocyte globulin-12

Ghrelin and some GHS treatment significantly attenuated CoCl(2)-induced hypoxic injury in H9c2 cells by increasing cell autophagy that in turn leads to better survival of cells through ROS inhibition, mTOR induction, and AMPK stimulation [76, 203, 204]. Ghrelin stimulated expression of genes including Beclin1, Atg12, Map1LC3A, and LC3 that triggered removal of dysfunctional mitochondria [59, 80] and finally activated cardiomyocyte AMPK [205], a pivotal regulator of cardiac autophagy [206].

Ionotropic action

Several in vivo and in vitro studies have suggested ghrelin as inotropic regulators in cardiomyocytes [207–209]. Cardiomyocytes express at least two subfamilies of functional K_{ca} channels [210–212]. The main current responsible for action potential duration is Ca^{2+}-independent transient outward K^+ current (I_{to}) [213, 214] and this Ca^{2+} influx through L-type Ca^{2+}channel opening/closing affects myocardial contractility [214]. Synthetic GHSs increased Ca^{2+}influx via voltage-gated Ca^{2+}channels in cultured neonatal as well as adult rat cardiomyocytes [215]. Similarly, ghrelin-stimulated Ca^{2+} influx leads to direct increase in Ca^{2+} channel conductance and/or decrease in K^+channel conductance has been decreased, resulting to prolonged depolarization [216]. However, it is difficult to establish whether this ionotropic regulation is directly involved in enhancement of cardiac output or not [43, 149]. Scientists' hypothesised ghrelin has negative inotropic and lusitropic effects via GHSR-1A but limited in right ventricle in vivo [216, 217]. It increased the amplitude of L-type Ca^{2+} transients and ICa, and L which trigger Ca^{2+}-dependent cellular processes that are abrogated by GHSR-1A blocking [218]. Ghrelin can regulate sarcoplasmic reticulum (SR) Ca^{2+} ATPase (SERCA2a); in turn, it induces calcium reuptake into the SR to increase cardiac relaxation [219]. Pretreatment with combination of apamin + ChTX, and K_{ca} channels blocker significantly attenuated the negative inotropic effect of ghrelin, both in normal and/or hypertrophic myocardium [216]. Ghrelin and hexarelin induced a transient increase followed by a reduction of contractile force and isolated ventricular myocyte shortening, Ca^{2+} transients and Ica in papillary myocytes in vitro [220]. This negative inotropic effect of hexarelin [221] was reduced by blocking nitric oxide (NO) synthesis [208, 222]. In contrast, hexarelin did not show any significant effect on calcium transients and L-type Ca^{2+} current (I_{Ca}), in isolated ventricular cells [134, 209]. Taken together, these findings indicate that the effects of hexarelin may act via nitric oxide (NO) release and prostacyclin (PGI2) from the endocardial endothelium, rather than direct effects on cardiomyocytes [209]. Infusion of ghrelin was also attenuated by inhibition of K_{ca} channels, suggesting that these channels may play critical role in ghrelin-induced vasodilation [223]. All these data provide further support that cardioprotection by ghrelin is mediated via inotropic effect and reinforcing the importance of I_{ca} and K_{ca}.

Anti-apoptotic pathways

Cardiomyocyte apoptosis is another major hallmark of cardiac diseases. The anti-apoptotic effects of acyl and des-acyl ghrelin have been confirmed in H9c2 cardiomyocytes and line HL-1 cell line through MAPK- and PI3K/Akt-dependent pro-surviving pathways via unknown n on-GHSR-1A receptor [29, 75, 224, 225]. Acyl-ghrelin prevents doxorubicin-induced cardiac intrinsic cell death by restoring IL-6/JAK2/STAT3 signalling and inhibition of STAT1 and/or by activating anti-oxidant enzymes superoxide dismutase and catalase [226, 227]. Ghrelin preserve mitochondrial membrane potential and energy metabolism. Furthermore, it upregulates anti-apoptotic proteins such as bcl-2 and inhibits cytC and the activation of the NF-kB pathway [227, 228]. Ang II-induced apoptosis of H9c2 cells is attenuated by regulating ER stress pathway [229], exerting a cardioprotective role against Ang II-induced cardiomyocytes apoptosis [230]. Obestatin, ghrelin gene-derived peptide, has also been reported to be anti-apoptotic in several cell types, including cardiomyocytes by PKC, PI3K, and/or ERK1/2 cascade, but mechanism of action is still a matter of conjecture [231]. Other than these pathways, ghrelin can also target miR-208, a crucial cardiac miRNA involved in the regulation of apoptosis [232].

Anti-inflammatory pathway

Ghrelin also exerts potent anti-inflammatory effects. T lymphocytes express both ghrelin and GHSR-1A, while ghrelin secretion is increased upon T cell activation; it exerts its effect by inhibiting IL-1β, IL-6, and TNF-α release in T cells and monocytes [233]; NF-κB, CD40, VCAM-1, and ICAM-1 is reduced in mice endothelial cells of blood vessels [165–168].

Interestingly, ghrelin can also counteract cytokine-induced apoptosis [60]. Same anti-inflammatory effect has been observed in endothelial cells through reducing NF-kβ activation by ghrelin and decreases inflammatory cytokines in response to endotoxin [168]. Ghrelin levels significantly decreased during sepsis, while exogenous administration in a rat model reduced organ injury by attenuating inflammation [70].

Autonomic nervous system regulation

Physiologically, ghrelin is permeable to blood-brain barrier (BBB) [234], acyl-ghrelin mainly can move brain-to-blood direction, and des-acyl ghrelin enters the brain by non-saturable transmembrane diffusion [235]. ICV microinjection of 1 nmol dose attenuated arterial pressure and heart rate as

sympatho-inhibitory response [151] in rabbits. Similarly, microinjections of ghrelin (20 pmol) into the nucleus of the solitary tract in anaesthetised rats (NTS) decreased arterial blood pressure and renal sympathetic nerve activity [129, 236]. Desacylated ghrelin acts via non-GHSR-1A receptor in bulbar nucleus of the brain [236]. Exogenous treatment of [D-Lys-3]-GHRP-6, a GHS-R1A antagonist inhibited by α- and β1-adrenoreceptor-mediated attenuation [237]. Ghrelin-mediated regulation of sympathetic nervous system, in turn, modulates vascular tone.

Vagal afferent nerve terminals are present in the heart and locally produced ghrelin can send signals to the NTS [44, 50]. Peripheral administered ghrelin suppresses LF/HF ratio and cardiac sympathetic nerve activity (CSNA) in animal model of MI [44, 51, 52]. MI induces CSNA, which had been prevented and decreased to pre-MI level when treated with ghrelin (150 μg/kg, sc) in rat [51]. Using a microdialysis technique, Shimizu et al. [238] demonstrated more directly that centrally administered ghrelin activated cardiac vagal nerve in anaesthetised rabbits. In ghrelin-KO mice, CSNA is disrupted, leading to arrhythmias after MI [50]. In addition, ghrelin can also modulate para-sympathetic nerve activity [71] as ghrelin administration at lateral cerebral ventricle did not alter dialysate norepinephrine concentrations but significantly increased dialysate acetylcholine concentrations [238]. Thus, ghrelin can inhibit excessive activation of CSNA, and in turn, activate cardiac parasympathetic activity to control heart (Fig. 4).

Age-related decline of ghrelin and association of cardiac complications

Most of the cohort studies revealed a reduction in circulating ghrelin in age-dependant manner [239–241]. Studies revealed significantly low circulating acyl-ghrelin levels and growth hormone secretion in the elderly [242, 243] although some showed no difference in ghrelin levels between young and elderly females [244]. Decline in ghrelin levels in elderly people aged 67–91 years during fasting, relative to young controls aged 27–39 years, has been reported [243]. Circulating desacyl ghrelin even improves prediction of cardiovascular disease in older hypertensive patients [245]. There is a significant negative correlation between three points, the mean fasting plasma ghrelin concentrations, BMI, and serum insulin levels in all groups of subjects [243]. However, the reduction in ghrelin levels was found to be independent of the differences in body composition or insulin resistance in this study [240].

Decline in gastric ghrelin mRNA levels in 19-month-old mice was observed than that of young ones [246]. Furthermore, fasting-induced increases in ghrelin were less robust in ageing comparable between 4 and 25 months of age [247]. In contrast, animal model studies in both Wistar and Lou C/Jall rats showed significant increase in ghrelin levels with ageing [248]. Similar increases in both total and active acyl-ghrelin were detected in C57BL/6J mice [249, 250]. Even Liu et al. [246] found no correlation between total

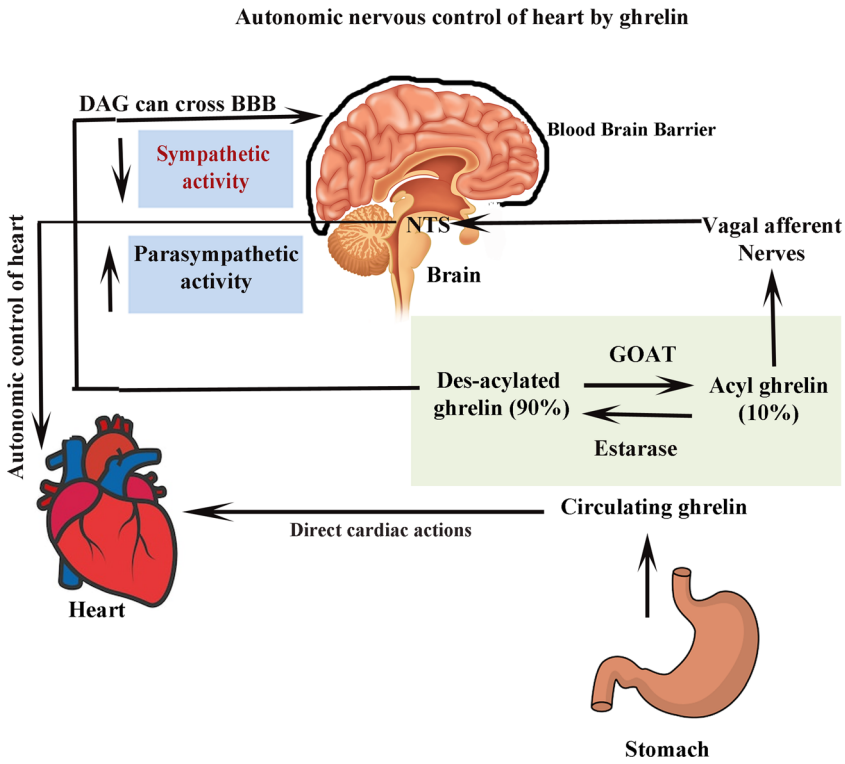

Fig. 4 Autonomic nervous control of the heart regulated by ghrelin. Schematic diagram showing cardiac vagal afferent nerve terminals send signals to nucleus of the solitary tract (NTS) that in turn control the vasomotor centre of the medulla. Ghrelin inhibits the sympathetic nerve activity, upregulates parasympathetic activity, and protects the heart from excessive damage

ghrelin and the age of C57BL/6 mice up to 6 months old. Increased ghrelin signalling promotes survival in mouse models of human ageing through activation of sirtuin1 [251]. Increased levels of plasma ghrelin have been proposed to be attributed to the decline of its receptor (and/or post-receptor) functions in senescent animals [250].

As we know, there are direct evidences of increased risk of different cardiovascular diseases in aged population [252]. More studies should be done if there is any direct effect of ghrelin- and age-dependant cardiovascular complications.

Clinical studies substantiate ghrelin to combat heart failure

The various experimental studies on cardiovascular effects of GHS and mode of actions have been described above in different headings to evaluate their potential role in the treatment of heart failure. However, very limited number of published data on human clinical trials is present till date. Infusion of ghrelin in low dose (0.1 μg/kg/min) for a short duration of 60 min in 12 CHF patients significantly increased mean arterial pressure, cardiac index, and stroke volume index without altering heart rate [189]. In another study, 10 patients with congestive heart failure were treated with ghrelin intravenously for 3 weeks, and LVEF and LV mass were significantly increased but LV end-systolic volume was decreased [47]. In addition, ghrelin treatment also helped to increase peak workload as well as peak oxygen consumption during extensive exercise, indicating improvement in systolic function and exercise capacity [47]. Recently, Sullivan et al. [253] described role of ghrelin/GHSR system before and after heart transplantation in human. Cardiac tissues were obtained from 10 patients undergoing cardiac transplant at the time of organ harvesting; GHSR and ghrelin both were strongly positively correlated in diseased heart, and both markers were negatively correlated with left ventricular ejection fraction [253]. In a randomized, doubleblind, and placebo-controlled crossover study, physiological increments of ghrelin concentration significantly increase left ventricular myocardial systolic velocity and endothelium-dependent vasodilatation in humans [41]. A large ghrelin bolus significantly increased stroke volume and decreased systemic vascular resistance and mean arterial pressure (MAP) in healthy volunteers [254], but in another study, a similar dose of ghrelin increased LVEF without change in MAP [193]. Not only in healthy volunteers, even in a pilot study with 62 patients of heart failure seems to be better with preserved ejection fraction with ghrelin treatment [255]. Higher and repeat dose infusions of ghrelin significantly decreased pulmonary capillary pressure along with mean arterial pressure and vascular resistance. Whereas, in same study, cardiac index, LVEF, and stroke volume have been increased significantly; these changes were associated with improved exercise capacity [47, 189]. In healthy men, intra-arterial ghrelin infusion leads to increased forearm vasodilation, independent of nitric oxide [152], and increase endothelium-dependent vasodilator responses in patients with metabolic disorders [123] by increasing availability of nitric oxide [256]. Randomized, blinded, and placebo-controlled unacylated ghrelin infusion protects endothelial cells and promotes for vascular remodelling in patients with type 2 diabetes mellitus [257]. But more long-term, multifactorial placebo-controlled human trials are required to establish therapeutic potential of ghrelin in CVD.

Conclusion and future direction

Ghrelin and its multiple physiological functions represent one of the extraordinary recent discoveries, initially concerning the energy balance regulation, but more recently, its cardioprotective effects are cropping up. Ghrelin and its receptors are widely distributed in cardiomyocytes as well as in blood vessels, including endothelial cells. Here, in this review, we tried to present a large body of recent homogeneous literature that demonstrated association of ghrelin with cardiovascular system along with its mechanism of actions. This miraculous peptide exerts cardioprotective effects, protection from ischemia/reperfusion injury, cardiac cachexia, cardiac hypertrophy and fibrosis, attenuation of left ventricular remodelling following myocardial infarction, improvement of left ventricular function, and cardiac capacity in patients with chronic heart failure. At the level of vasculature, it also plays a pivotal role on endothelial function, in particular, anti-oxidant, anti-inflammatory, and anti-apoptotic effects by improving NO availability and restoring the endothelin-1/nitric oxide imbalance. At higher doses, it also decreases blood pressure, by modulating sympathetic nervous system. In particular, these beneficial cardiac effects and vascular protection indicate that ghrelin is a potent candidate for the treatment of congestive heart failure and should be explored more for human welfare. Synthetic ghrelin that mimic the ghrelin-related effects and can target its receptors is well established and used in metabolic disorders and obesity treatment. But this peptide can act as GH-independent pathway too in cardiomyocytes and that is much neglected in science till date. Therefore, more studies are encouraged to use ghrelin as a potent CVD drug from bench to bedside.

Authors' contribution SG conceived the idea and prepared the manuscript as well as the figures. AM edited the draft manuscript and checked and arranged the references. Both the authors approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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