Ghrelin and Cardiovasculature

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Abstract

Apart from stimulating growth hormone (GH) secretion and a regulatory role for appetite and metabolism, it has become increasingly clear that GH secretagogues (GHS) and ghrelin exert a number of effects on the cardiovascular system. The main cardiovascular actions of GHS are possible inotropic effects, vasodilation, reported cardioprotective effects against ischaemia and *in vitro* effects on cardiomyocytes involving cell proliferation and antiapoptotic actions. An interesting and intriguing feature of the cardiovascular effects of GHS is that they may be exerted directly on the heart and vasculature rather than being mediated by GH. Evidence to suggest this is the finding of GHS binding sites on cardiomyocytes and the fact that some of the effects of GHS can be expressed also in the absence of GH. Although these results offer interesting possibilities for ghrelin and/or GHS to be used as therapeutic tools in cardiovascular disease, larger clinical trials in this area are still lacking. Future studies aiming at evaluating a role of GHS and ghrelin in the treatment of cardiovascular disease are warranted.

Keywords

Ghrelin, growth hormone secretagogues, heart, cardiovascular

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Growth hormone secretagogues (GHS) were originally devised as small synthetic compounds, either peptides or non-peptides, with the ability to stimulate GH secretion from the pituitary.[1] It has been demonstrated that GHS signalling is mainly through the GH secretagogue receptor (GHS-R), which is a G-protein-coupled receptor with seven transmembrane domains.[2] Studies using *in situ* hybridisation have revealed expression in the pituitary, hypothalamus and hippocampus and the identification of this orphan receptor triggered an active search for the natural ligand.[3] A couple of years later, the endogenous ligand for the GHS-R was purified from the rat stomach and named ghrelin.[4] Ghrelin was identified as a 28-amino acid peptide with an n-octanoylation of the serine-3 residue, and this quite unique feature has been found to be critical for the classic effects of ghrelin.[4] The highest level of ghrelin expression is in the gastric mucosa, although expression at low levels has been found in multiple organs and tissues.[4] Apart from the ability to stimulate GH secretion and to exert effects regulating appetite and metabolism, it has become increasingly evident that GHS and ghrelin have a number of effects on the cardiovascular system. This overview of the main cardiovascular actions of GHS and ghrelin will focus on possible inotropic effects, vasodilation, anti-inflammatory properties, reported cardioprotective effects against ischaemia and *in vitro* effects on cardiomyocytes involving cell proliferation and antiapoptotic actions. An interesting and intriguing feature of the cardiovascular effects of GHS is that they may be targeted directly on the heart and vasculature rather than being mediated by increased GH secretion. Evidence to support this is the finding of GHS binding sites on cardiomyocytes and the fact that some of the effects of GHS can be expressed also in the absence of GH. Finally, the potential use of GHS and ghrelin as therapeutic agents in heart failure and related cardiac cachexia will be discussed.

Inotropic Effects

Inotropic effects of GHS during physiological conditions have been suggested. However, it cannot be ruled out that the observed improvement in contractility is either secondary to vasodilatory effects or secondary to increased secretion and action of GH. However, a few studies suggesting inotropic effects deserve attention since improvement of systolic function was reported in the absence of decreased peripheral resistance. An increased left ventricular ejection fraction (LVEF) in both healthy volunteers[5] and patients with GH deficiency (GHD)[6] was reported within 15 minutes of intravenous (IV) hexarelin injection, a peptidic GHS, without a concomitant lowering of blood pressure. In a more recent study, ghrelin was administered subcutaneously (SC) to six healthy volunteers.[7] Ghrelin significantly increased LVEF and end-systolic volume 30 minutes after administration without affecting blood pressure or heart rate. Currently, there is no known mechanism for the inotropic actions of GHS, and experimental data addressing this topic are lacking. However, some of the positive effects on contractility, at least in non-GHD conditions, may be explained by increased GH secretion, which has been demonstrated to have inotropic effects in experimental models, possibly related to alterations in Ca^{2+} transients.[8]

Vasodilation

A second characterised cardiovascular action of GHS is a vasodilatory effect observed in both clinical and experimental studies. In a study

by Kangawa and collaborators, a single injection of ghrelin caused a significant decrease in blood pressure.[9] In unpublished observations from our own laboratory, we also measured a modest but significant decrease in systolic blood pressure in hypophysectomised (hx) rats treated with ghrelin for two weeks. However, the vasodilatory effect of ghrelin and GHS is not entirely consistent in the literature, as described above.[5–7] This may possibly be due, at least in part, to differences in administration modes and dosing.

Several possible mechanisms for the vasodilatory effect of GHS have been suggested. A recent study suggests an antagonistic action on the vasoconstrictor effect of endothelin-1 in mammary arteries.[10] Studies on aortic ring preparations from GH-deficient rats treated with ghrelin showed an increased maximal acetylcholine-induced vasorelaxation compared with placebo, and this vasodilatory effect was accompanied by an increase in endothelial nitric oxide synthase (eNOS) production.[11] Moreover, the treatment effect of ghrelin could be inhibited by N(G)-nitro-L-arginine methyl ester, a non-selective NOS inhibitor, adding further support for a nitric-oxide-dependent pathway. However, the same research group has also reported possible vasodilatory effects independent of nitric oxide mechanisms.[12] Another interesting possible mechanism that has been reported is a central effect, involving decreased sympathetic nervous activity and arterial pressure, observed after unilateral microinjection of ghrelin into the nucleus of the solitary tract of rats.[13]

These findings are in line with a previous report describing decreased mean arterial blood pressure and sympathetic nerve activity by ghrelin.[14] Additional support for this concept can be found in a recent study performed on conscious rats using the ghrelin antagonist (GhA) [d-Lys-3]-GHRP-6. Administration of this GhA revealed a dose-dependent increase in mean arterial pressure and heart rate that could be abolished by parallel sympathetic blockade of alpha- and beta-adrenoreceptors.[15]

Anti-inflammatory Effects

Apart from vasodilatory effects, ghrelin may also have other vasoactive and anti-inflammatory properties. It has been shown that ghrelin inhibits cytokine release, activation of nuclear factor-κB and mononuclear cell binding in cultured human umbilical vein endothelial cells (HUVEC), which could potentially be important should GHS be considered as therapeutic agents in conditions with atherosclerosis.[16] It has also been reported that exogenous ghrelin inhibited synthesis of the pro-inflammatory cytokines interleukin (IL)-1β and tumour necrosis factor (TNF)-α in lipopolysaccharide (LPS)-stimulated murine macrophages.[17] Additional evidence for anti-inflammatory effects of ghrelin include the findings that T lymphocytes express both ghrelin and the GHS-R and that secretion of ghrelin is increased when the T lymphocytes are activated.[18] This may suggest a modulatory role on the immune system, since ghrelin was also reported to inhibit IL-1β, IL-6 and TNF-α in these cells.[18]

Cardioprotective Effects Against Ischaemia

Accumulating evidence from several research groups using different experimental models supports cardioprotective effects of GHS against ischaemia. In one of the first studies addressing this topic, De Gennaro Colonna and collaborators used antiserum to GHRH in order to induce GH deficiency (GHD) in rats and then treated them with GH or hexarelin for two weeks.[19] After killing the rats their hearts were subjected to retrograde aortic perfusion under ischaemic conditions. Control rats with GH deficiency showed marked increase in left ventricular end-diastolic pressure (LVEDP) and poor recovery of contractility after reperfusion. By contrast, rats treated with GH or hexarelin had normalised cardiac function. In a further study from the same research group, hx rats were treated with GH or hexarelin, which again protected the hearts from cardiac damage during ischaemic reperfusion *in vitro*.[20] Interestingly, both of these studies suggest effects independent of increased GH secretion since the cardiovascular effects of hexarelin were expressed in two different models of GHD.

Myocardial stunning is a clinical entity that can be defined as transient myocardial ischaemia followed by reperfusion, which causes a reversible cardiac dysfunction involving both systolic and diastolic function. A rabbit model has been used to study possible protective effects of GH and GHS on myocardial stunning.[21] Animals were pre-treated with GH or GHRP-2 for two weeks. Subsequently, hearts were blood-perfused and subjected to 15 minutes of ischaemia followed by 80 minutes of reperfusion and compared with non-ischaemic hearts. In this study there were no significant effects of either GH or GHRP-2 on systolic function. However, a significant improvement of diastolic function after pre-treatment with GHRP-2 was observed, suggesting a novel therapeutic possibility for myocardial stunning.

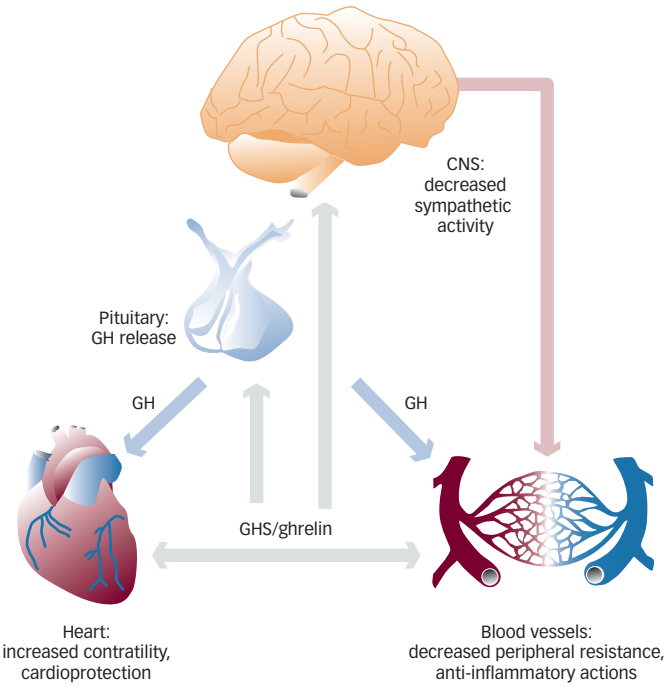
Experimental Models of Congestive Heart Failure

In a previous study by our laboratory, an experimental approach was used.[22] Intact rats were subjected to experimental myocardial infarction by coronary artery ligation, and after four weeks of recovery, the rats were treated with two doses of hexarelin 10 or 100µg/kg/day, GH 2.5mg/kg/day or saline for two weeks. Cardiac structure and function were evaluated with echocardiography at baseline and at the end of the experiment. Stroke volume was significantly increased in both the high-dose hexarelin group and the GH group compared with control rats. Similar findings were also seen regarding cardiac output, which was also increased by hexarelin and GH. Both of these parameters remained significantly elevated when normalised to bodyweight. Interestingly, only GH but not hexarelin treatment significantly increased bodyweight and kidney insulin-like growth factor I (IGF-I) messenger RNA (mRNA), suggesting that the effects of hexarelin were independent of GH.

As an alternative method to coronary artery ligation, electric pacing of the heart has been developed as a model for impairing cardiac function. King and collaborators induced rapid pacing at 24bpm for three weeks in pigs.[23] An orally bioavailable GHS termed CP-424,391 was administered to one group of animals at initiation of pacing. At the end of the experiment after three weeks of pacing and treatment, GHS-treated pigs had significantly higher fractional shortening and lower wall stress due to cardiac hypertrophy compared with control animals. Serum IGF-I was increased approximately two-fold, which would suggest an effect at least partially mediated by an increased GH secretion.

Only a limited number of published studies so far have used ghrelin rather than synthetic GHS to study effects after myocardial injury. Kangawa and collaborators used rats with experimental myocardial infarction and treated them with ghrelin 100µg daily for three weeks.[24] Echocardiography and catheterisation were used to evaluate effects

Figure 1: Schematic Illustration of Possible Interactions Between Growth Hormone Secretagogues/Ghrelin, Central Nervous System and the Cardiovascular System



Exogenous growth hormone (GH) secretagogues (GHS) or ghrelin can increase pituitary GH release leading to GH-mediated effects on the heart or the vasculature. Alternatively, GHS or ghrelin can act directly via binding sites in the cardiovascular system, promoting decreased peripheral resistance and cardioprotection. Anti-inflammatory effects on vascular cells and improved cardiac contractility have also been suggested as possible GHS/ghrelin effects. Interactions between ghrelin and the central nervous system (CNS) resulting in decreased sympathetic activity have also been proposed.

of ghrelin showing higher cardiac output and fractional shortening in ghrelin-treated rats compared with controls. Another study using echocardiography before and after four weeks of ghrelin administration to MI rats revealed improved systolic function and decreased LV remodelling.[25] Moreover, this improvement in function and structure was accompanied by a decreased inflammatory response and expression of metalloproteinases.[25] Ghrelin has also been reported to be protective when added *in vitro* during ischaemic reperfusion of isolated rat hearts.[25]

Differential Effects of Growth Hormone Secretagogues and Ghrelin

An interesting aspect that has been suggested is the possibility that cardioprotective effects may not be completely interchangable between different GHS and ghrelin. Torsello and collaborators recently suggested that cardioprotective effects against ischaemia after pre-treatment with hexarelin could not be replicated by ghrelin.[27] In this study, hx rats were pre-treated for seven days with either hexarelin or ghrelin and their hearts were subsequently subjected to ischaemia/reperfusion *in vitro*. During ischaemia there was a gradual increase of LVEDP pressure in hearts subjected to ischaemia compared with controls. Pre-treatment with hexarelin but not ghrelin partially normalised LVEDP and protected the hearts from injury. It could be speculated that this differential effect of hexarelin and ghrelin might be in part due to different affinities to a variety of subtype GHS receptors.

An intriguing possibility that some of the cardiovascular effects of GHS may be mediated through the CD36 receptor was recently suggested.[28] The CD36 receptor is a multifunctional B-type scavenger receptor and binding of hexarelin was found to occur in cardiomyocytes to a membrane protein identical to rat CD36 receptor. One suggested GHS effect associated with activation of the CD36 receptor was coronary vasoconstriction.[28] This is intriguing and in contrast to otherwise reported vasodilatory effects of GHS. Whether the coronary vasoconstriction is specifically mediated by the CD36 receptor is not known, and more studies and better understanding of GHS binding sites are needed to clarify this.

So to summarise, accumulating evidence suggests beneficial effects of GHS on both diastolic and systolic function after ischaemic injury, and this has been shown in several experimental models and species and to a limited extent in short-term clinical trials. Moreover, these effects are present also in the absence of GH, suggesting direct interaction between GHS and the cardiovascular system.

Proliferative, Antiapoptotic and Metabolic Effects on Cardiomyocytes

The possibility of GH-independent effects of GHS has prompted several studies on cultured cardiomyocytes to elucidate possible effects and signalling. In studies from our own laboratory, we have found that hexarelin and ghrelin increase thymidine incorporation, indicating increased proliferation of H9c2 cardiomyocytes in a dose-dependent and specific manner.[29] Moreover, binding studies on cardiomyocyte cell membranes revealed specific binding in the absence of detectable expression of the GHS-R1a,[29] which is the classic cloned G-protein-coupled receptor expressed mainly in the pituitary and the hypothalamus.[2] This would suggest an alternative binding site in the heart.[29]

Possible antiapoptotic effects of GHS have also been studied on cardiomyocytes *in vitro*, and cell survival signalling has been described.[27] Both ghrelin and desoctanoyl ghrelin had similar antiapoptotic effects on primary cardiomyocytes, H9c2 cardiomyocytes and endothelial cells with signalling via MAPK and PI3K/Akt-dependent pathways.[30]

In a recently published study, hexarelin was found to decrease apoptosis induced by angiotensin-II in cultured rat cardiomyocytes.[31] Using the Tunnel assay, hexarelin was found to significantly decrease apoptosis and to inhibit caspase-3 activity and Bax expression. Moreover, expression of Bcl-2 was increased, providing evidence for antiapoptotic effects of hexarelin through well-established antiapoptotic signalling. Thus, *in vitro* findings would support both proliferative and antiapoptotic effects of GHS involving signalling via well-established cell survival pathways.

Another potentially important effect of ghrelin on cardiomyocytes was recently reported and involves stimulation of 5'-AMP-activated protein kinase (AMPK).[32] This kinase has a central role in regulating energy supplies in cells and is activated in anaerobic conditions. Thus, a beneficial effect of ghrelin on cellular energy supply may contribute to the mechanism of cardioprotection.[33]

Clinical Use of Ghrelin and Growth Hormone Secretagogues in Heart Failure

The experience from clinical trials with GHS and ghrelin in conditions with impaired cardiac function is also very limited. Acute administration of hexarelin increased LVEF in patients with ischaemic but not dilated cardiomyopathy.[34] A more recent clinical trial with acute

administration of ghrelin to patients with congestive heart failure demonstrated beneficial effects.[35] Twelve patients with CHF were given a single IV infusion of human ghrelin or placebo. Ghrelin significantly increased cardiac index and stroke volume index and decreased systemic vascular resistance within 60 minutes. Moreover, in anaesthetised patients undergoing bypass surgery, short-term hexarelin infusion was found to increase LVEF, cardiac output and mean arterial pressure without any changes in peripheral resistance.[36] Only one study with longer duration of treatment of patients with congestive heart failure has been published so far.[37] In this open study, 10 patients with congestive heart failure of heterogeneous aetiology were treated with IV ghrelin for three weeks. Ghrelin significantly increased LVEF, LV mass and LV end-systolic volume. Moreover, ghrelin also increased peak workload and peak oxygen consumption during exercise and there was a reported decrease in plasma norepinephrine. No serious adverse events were observed.

End-stage CHF is sometimes associated with cachexia, which is a severe catabolic state characterised by weight loss and muscle wasting. Thus, it is tempting to speculate that ghrelin and GHS may have multiple beneficial effects on this patient population by exerting both positive cardiovascular and orexigenic actions. To date, there are no long-term clinical trials substantiating this hypothesis, although preliminary data suggest that administration of ghrelin increases muscle strength and lean body mass in patients with CHF.[37] Whether GHS and ghrelin will find a role as therapeutic agents in the treatment of congestive heart failure remains to be further investigated in more long-term and placebo-controlled trials. However, the clinical studies published to date certainly open interesting possibilities. In relation to established agents used for treatment of CHF, GHS and ghrelin may have additional potentially beneficial properties such as orexigenic, anti-inflammatory and antiapoptotic effects and a regulatory role regarding myocardial energy supplies.

Conclusion

Although initially recognised for its GH-releasing properties, the cardiovascular system has been recognised as potentially important target for GHS. Moreover, a limited number of studies also indicate cardiovascular effects of ghrelin. So far, reported cardiovascular effects of GHS and/or ghrelin include lowering of peripheral resistance, anti-inflammatory effects, possible improvement of contractility and cardioprotective effects both *in vivo* and *in vitro* (see *Figure 1*). Moreover, central effects effects by ghrelin, possibly mediated in part by the sympathetic nervous system, may also affect the cardiovascular system.

Taken together, these results offer an interesting perspective on the future where further studies aiming at evaluating a therapeutic role for GHS and ghrelin in the treatment of cardiovascular disease are warranted. ■

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