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Ghrelin, a growth hormone–releasing peptide that was first discovered in the stomach of rats in 1999, is an endogenous ligand of growth hormone secretagogue receptors (GHSRs).[1] Through binding to its receptors in the brain, ghrelin was initially shown to strongly stimulate the release of growth hormone and promote food intake.[2] Subsequent studies revealed that GHSRs are expressed ubiquitously in many organs and tissues, and ghrelin functionally participates in the regulation of diverse processes including appetite control, energy balance, body weight maintenance, glucose and fat metabolism, cell proliferation, and apoptosis, as well as the modulation of gastrointestinal, cardiovascular, pulmonary, and immune functions.[3,4] The primary receptor of ghrelin is currently thought to be GHSR1a; however, other unidentified receptors might exist.[5,6] The high expression of GHSR1a in the heart and large vessels provides evidence of its cardiac activity,[7] indicating ghrelin is a promising new therapeutic agent for cardiovascular diseases. In this review, we discuss some of the characteristic features of ghrelin treatment and its possible therapeutic roles in animals and patients afflicted with common cardiovascular diseases.

Production, Acylation, and Regulation

Ghrelin is produced predominantly in the stomach and is secreted from the submucosal layer into the bloodstream but not into the gastrointestinal tract. In situ analyses revealed that ghrelin and its mRNA are mainly localized in X/A-like cells, a major endocrine population in the gastric oxyntic mucosa that are morphologically similar to pancreatic α cells.[1] Cells that produce low levels of ghrelin are also found in the lung, bowel, pancreas, kidney, placenta, testis, hypothalamus, pituitary gland, and the immune system.[8–18] Ghrelin is reportedly produced in neoplastic tissues such as gastric and intestinal carcinoids and medullary thyroid carcinomas.[19–21] In addition to endogenous ghrelin, growth hormone secretagogues, a heterogeneous group of synthetically produced peptides and nonpeptides, have been developed as ghrelin alternatives that can bind GHSRs and stimulate growth hormone secretion in both animals and human subjects.

The predominant active form of human ghrelin is a 28-aa peptide that is octanoylated at the serine-3 position by *n*-octanoic acid. This acylation of ghrelin is essential for it to bind to GHSR1a, which in turn is required for its growth hormone–releasing activity and most likely for its other endocrine actions as well.[22–25] However, nonacylated ghrelin is present in human serum in far greater quantities than acylated ghrelin. In healthy adults, the plasma concentration of acylated ghrelin is 10 to 20 fmol/mL, whereas total ghrelin is 100 to 150 fmol/mL.[26] Although nonacylated ghrelin does not bind to and activate GHSR1a and thus seems to be devoid of any endocrine activity, some studies reported that it exerts some nonendocrine activity including cardiovascular and antiproliferative effects, probably by binding to a different receptor family.[9,27] Both ghrelin and nonacylated ghrelin can inhibit apoptosis in primary adult cardiomyocytes, H9c2 cardiomyocytes, and endothelial cells in vitro through activating extracellular signal–regulated kinase 1/2 and Akt serine kinase.[6] Furthermore, ghrelin and nonacylated ghrelin recognize common high-affinity binding sites on H9c2 cardiomyocytes, which do not express GHSR1a.[6] Controversially, in isolated rat hearts subjected to 30 minutes of ischemia followed by 120 minutes of reperfusion, triphenyltetrazolium chloride staining shows that ghrelin significantly reduces infarct size, whereas nonacylated ghrelin has no such cardiovascular effect.[28]

The regulation of ghrelin by acute feeding and chronic energy balance is regarded as an adaptive physiological response.[29] The level of ghrelin mRNA in the stomach is increased by fasting and decreased by feeding.[30,31] Furthermore, circulating ghrelin levels are decreased in obese individuals[30,32–34] and postprandially.[35] In contrast, ghrelin is increased in lean people, in patients with anorexia nervosa,[36–39] preprandially,[31] and during conditions of food deprivation.[40,41] Patients with noncachectic chronic heart failure have normal levels of ghrelin, and the plasma ghrelin level is significantly higher in chronic heart failure patients with cachexia.[42] If rats with chronic heart failure are treated with ghrelin, appropriate weight gain and muscle/bone ratios can be maintained.[37]

Ghrelin Receptors

Two cDNAs, GHSR1a and GHSR1b, have been identified and are generated by alternative splicing of a pre-mRNA.[43–45] The GHSR1a cDNA encodes a receptor comprising 366 amino acids and 7 transmembrane domains, whereas the GHSR1b cDNA encodes a shorter form of the receptor, consisting of 289 amino acids and only 5 transmembrane domains.[44] The binding of ghrelin and growth hormone secretagogues to GHSR1a activates the phospholipase C signaling pathway, leading to an increase in inositol phosphate turnover and protein kinase C activation and the subsequent release of Ca^{2+} from intracellular stores.[45,46] GHSR1a activation also inhibits K^+ channels,

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allowing the entry of Ca^{2+} through voltage-gated L-type channels.[47,48] Unlike GHSR1a, GHSR1b fails to bind and respond to growth hormone secretagogues,[44] and its functional role remains to be defined. Therefore, GHSR1a is thought to be the primary ghrelin receptor. Data indicate the existence of several other receptors, but they have not yet been identified.[5,6]

Studies focusing on the distribution of GHSRs showed a notable concentration of these receptors in the hypothalamus–pituitary region, consistent with its role in regulating growth hormone release.[44,49–51] More recent localization studies have demonstrated that GHSRs are expressed in multiple peripheral organs and tissues such as the heart and aorta,[42,52,53] stomach and intestine,[9] pancreas,[49] kidney,[10] as well as in different human pituitary adenomas[12,54] and endocrine neoplasms of the lung,[55] stomach,[19] and pancreas.[13,54,56] Notable, however, the primers used for reverse transcription polymerase chain reaction are generally not designed to differentiate GHSR1a from GHSR1b. These data are in accordance with those which indicate that ghrelin has broader functions beyond the control of growth hormone release and food intake.

Cardiovascular Activity

GHSR1a mRNA has been shown to be present in the heart and aorta,[52,57] cultured cardiomyocyte cell line, and human vascular endothelial cells.[58] Specific binding sites for ghrelin have been identified in rat hearts and human arteries, where the density of ghrelin receptors is upregulated in response to atherosclerosis.[59] Using immunofluorescence analysis, we showed the existence of GHSR1a in the myocardia of rats and mice. Costaining with acetylcholine esterase and choline acetyltransferase suggested that GHSR1a is localized on or in close proximity to the vagal nerve terminals in the heart.[60,61] Therefore, substantial evidence indicates that ghrelin has a cardiovascular function.

In healthy subjects, the administration of ghrelin reportedly dilates human arteries, decreases blood pressure, reduces cardiac afterload, and increases cardiac output.[52,62,63] In addition, ghrelin potently improves energy balance, modulates the autonomic nervous system, and directly acts on cardiomyocytes, effects that are all beneficial for cardiovascular disease in animals and patients. These studies highlight the therapeutic potential of ghrelin in heart failure and myocardial infarction (MI).

Modulation of Autonomic Nervous Activity

Central Neural Modulation

Considerable data on the potential effects of ghrelin on autonomic nervous modulation have been amassed.[64–68] The receptor for ghrelin has been shown to localize in the main cardiovascular control centers in neurons of the nucleus tractus solitarius, and central administration of ghrelin attenuates renal and adipose tissue sympathetic nervous activity (SNA).[64,65,68] Furthermore, when ghrelin is microinjected into the nucleus of the solitary tract, the region of the brain that is important for controlling the autonomic nervous system, significant decreases in heart rate and mean arterial pressure are observed.[65]

Peripheral Neural Modulation

Ghrelin receptors have been found on the vagal afferent terminals in the stomach and heart,[60,61,69] and peripheral administration of ghrelin attenuates sympathetic tone and the cardiac efferent firing rate.[60,61,70] Ghrelin receptors are synthesized in vagal afferent neurons and are transported to the afferent terminals in the stomach. Ghrelin produced in the stomach stimulates the gastric vagal afferent nerve that leads to the nucleus tractus solitarius, influencing its neuronal activity and increasing feeding behavior.[69] Blocking the gastric vagal afferent nerve by ligating the gastric vagal branch abolishes ghrelin-induced feeding, growth hormone secretion, and activation of the neurons that produce neuropeptide Y and growth hormone–releasing factor.[69] In another work, ghrelin signaling in the nucleus tractus solitarius was shown to decrease the presynaptic release of glutamate at the terminals of vagal afferents.[71] We showed that GHSR1a is present in the myocardia of rats and mice, where it is localized at or in proximity to vagal nerve terminals in the heart.[60,61] Blocking the vagal afferent nerves by pretreating them with capsaicin, a toxin specific for sensory afferent neurons, markedly attenuates the beneficial effects of ghrelin treatment after MI, such as the inhibition of cardiac SNA and arrhythmias and the improvement of prognoses.[61] Similarly, the sympathoinhibitory effect of intravenous ghrelin administration in postgastrectomy vagotomized patients is abolished, suggesting that vagal sensory afferents mediate the effects of peripheral ghrelin.[72] Taken together, these data suggest that peripheral ghrelin acts on the vagal afferent nerves that send projections to the nucleus tractus solitarius, resulting in a decrease of cardiac SNA.[73]

Neurotransmitters

Other evidence of its modulation of autonomic nervous activity comes from ghrelin-regulating neurotransmitters. Subcutaneous administration of ghrelin has been shown to suppress MI-induced increases in plasma norepinephrine concentration.[60] Furthermore, blood norepinephrine and epinephrine levels are increased in response to chronic administration of the ghrelin receptor antagonist [D-Lys^3]-GHRP-6.[74] In ghrelin knockout mice, the plasma norepinephrine concentration is ≈6-fold higher than in wild-type mice 30 minutes after MI.[61] Two weeks after MI, the plasma epinephrine concentration is still significantly higher in ghrelin knockout mice than in wild-type mice, whereas both metoprolol and ghrelin treatments significantly decrease the elevated levels of epinephrine and norepinephrine in ghrelin knockout mice.[75]

Direct Activity on Cardiomyocytes

In several studies, the effects of exogenous ghrelin were assessed directly in cultured cardiomyocytes or isolated working hearts, rather than on the autonomic nervous system.[6,58,76–80] Perfusion of ghrelin after ischemia was shown to produce a positive inotropic effect on ischemic cardiomyocytes through activating the GHSR1a receptor and protein kinase C signaling cascade, protecting them from ischemia–reperfusion injury.[58,78] Through activating GHSR1a, ghrelin can effectively preserve the electrophysiological properties of cardiomyocytes after ischemia–reperfusion injury, inhibiting cardiomyocyte apoptosis and promoting cell survival.[79] In addition, both acylated and nonacylated ghrelin are able to prevent cell death in cultured H9c2 cardiomyocytes and endothelial cells that have been induced by doxorubicin, serum withdrawal, or activation

by Fas ligand.[6,80] These effects are possibly mediated through binding an unidentified receptor and activating extracellular signal–regulated kinase 1/2 and Akt serine kinase.[6] However, ghrelin has also been reported to negligibly protect isolated working hearts from ischemia in rats.[81]

Ghrelin as a Therapeutic Agent

Heart Failure

In rats with chronic heart failure, ghrelin treatment attenuates the development of left ventricle (LV) remodeling and improves LV dysfunction as indicated by the increases in cardiac output and LV fractional shortening.[42] Ghrelin treatment was also associated with a reduction in systemic vascular resistance, likely reflecting a decrease in the afterload.[37] Therefore, ghrelin has been proposed as therapy for patients with heart failure. In fact, ghrelin administration significantly decreases systemic vascular resistance and increases the cardiac and stroke volume indexes of patients with chronic heart failure.[52,63] Furthermore, intravenous administration of ghrelin (2 μg/kg BID for 3 weeks) significantly improves LV ejection fraction from 27% to 31% and increases peak workload and oxygen consumption during exercise while dramatically decreasing plasma norepinephrine from 1132 to 655 pg/mL.[82] Interestingly, these beneficial effects of ghrelin on heart failure are also observed in hypophysectomized rats,[37] suggesting it has a minor role in the hypothalamus–pituitary axis.

Myocardial Infarction

The cardioprotective effects of exogenous ghrelin administration on heart function have also been demonstrated in MI models. In rats, LV enlargement induced by acute MI is significantly attenuated by ghrelin treatment (100 μg/kg BID for 2 weeks), substantially decreases LV end-diastolic pressure, and improves cardiac function, as indicated by d*P*/d*t*max and d*P*/d*t*min values.[60] The increase in the morphometric collagen volume fraction in the noninfarct regions is also reduced, and collagen I and III mRNA levels are decreased by ghrelin treatment.[60] Notably, the infarction-induced increases in heart rate and cardiac SNA are suppressed completely in ghrelin-treated animals.[60] Furthermore, ghrelin administration prevents arrhythmias and reduces mortality in the acute phase after MI. Using a neural recording technique, it has been shown that early intervention by using one bolus of ghrelin prevents the increase in cardiac SNA after acute MI. As a result, the ghrelin-treated group was shown to have fewer arrhythmic insults 2 to 3 hours after MI.[70] Ghrelin administration also significantly decreases the high mortality rate after MI (61% in saline-treated versus 23% in ghrelin-treated rats).[70]

In ghrelin knockout mice, cardiac SNA, represented by the ratio of low- to high-frequency power in heart rate variability analyses, is markedly increased 18-fold over that observed in wild-type mice 30 minutes after MI, resulting in a high incidence of malignant arrhythmias and mortality in the acute phase.[61] Subcutaneous supply of exogenous ghrelin decreases activated cardiac SNA and reduces the incidence of malignant arrhythmias and mortality.[61] Similarly, metoprolol treatment in ghrelin knockout mice is associated with low mortality resulting from malignant arrhythmias during the acute phase, further demonstrating that the origin of these arrhythmias relates to the imbalance in cardiac SNA.[75] Activation of cardiac SNA, deterioration of heart function, and severe remodeling are also manifested in ghrelin knockout mice 2 weeks after MI, accounting for the high mortality, particularly in cases that have been caused directly by heart failure.[75] Chronic treatment with metoprolol or ghrelin, which is associated with cardiac SNA inhibition and a decrease in plasma catecholamine levels, improves heart dysfunction, remodeling, and mortality in ghrelin knockout mice.[75] Taken together, these findings indicate that both exogenous and endogenous ghrelin are crucial in balancing the autonomic nervous system, preventing the incidence of arrhythmias, protecting cardiac function, and improving remodeling and prognoses after acute MI.

Conclusions

As described above, existing evidence supports that ghrelin can serve as a novel medication for cardiovascular diseases. Because ghrelin is an endogenous hormone, it may be advantageous over other drugs with respect to tolerance. We have shown that the therapeutic dosage of ghrelin has little influence on baseline blood pressure, heart rate, and cardiac SNA.[61] In conscious rats after MI, acute administration of ghrelin decreases the activated low- to high-frequency power ratio; however, in sham-operated rats, the low- to high-frequency power ratio and heart rate are not substantially affected by ghrelin administration.[60] Similar phenomena are observed in humans; although constant infusion of the physiological dose of ghrelin for 1 hour slightly reduces blood pressure and increases muscle sympathetic nervous activity at rest, it significantly blunts the cardiovascular and sympathetic responses to mental stress in lean and obese individulas.[83] These findings indicate that ghrelin has a stronger effect on the activated sympathetic nervous system than on the nonactivated system.[60] Thus, ghrelin seems to be a relatively safe therapeutic agent for the treatment of cardiovascular diseases.

However, ghrelin can act on GHSRs in the central nervous system to promote feeding and adiposity[40,84] and also act on GHSRs in the pancreas to inhibit glucose-stimulated insulin secretion.[85] Long-term administration of ghrelin might promote weight gain and impair glucose tolerance and therefore be contraindicated for uncontrolled obesity and diabetes mellitus. Furthermore, ghrelin is an unstable natural peptide that is easily transformed and degraded, potentially limiting its clinical use.

In summary, ghrelin administration has potent beneficial effects on cardiovascular diseases. such as heart failure, MI, and fatal arrhythmias, through various mechanisms including direct actions on cardiovascular cells and modulation of the autonomic nervous system. These results suggest the potential suitability of ghrelin as a new therapeutic agent for cardiovascular diseases. Additional studies elucidating the clinical efficacy and safety, as well as the contribution of each mechanism to the beneficial impact of ghrelin, are needed.

Disclosures

None.

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