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The cardiovascular action of hexarelin

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Abstract

Hexarelin, a synthetic growth hormone-releasing peptide, can bind to and activate the growth hormone secretagogue receptor (GHSR) in the brain similar to its natural analog ghrelin. However, the peripheral distribution of GHSR in the heart and blood vessels suggests that hexarelin might have direct cardiovascular actions beyond growth hormone release and neuroendocrine effects. Furthermore, the non-GHSR CD36 had been demonstrated to be a specific cardiac receptor for hexarelin and to mediate its cardioprotective effects. When compared with ghrelin, hexarelin is chemically more stable and functionally more potent. Therefore, it may be a promising therapeutic agent for some cardiovascular conditions. In this concise review, we discuss the current evidence for the cardiovascular action of hexarelin.

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1 Introduction

Growth hormone secretagogues (GHS) are a class of small synthetic peptides that stimulate growth hormone (GH) release through binding to the growth hormone secretagogue receptor (GHSR) 1a. Moreover, GHSR 1a is a G-protein-coupled receptor originally identified in the hypothalamus and pituitary,[1] and later recognized as the receptor for the endogenous hormone ghrelin.[2] The peripheral distribution of GHSR 1a in the heart, adrenals, fat, prostate, bone, and digestive tract has supported physiological roles of GHSs and ghrelin independent of GH release and neuroendocrine stimulation. For example, GH-independent effects on orexigenic properties, fat metabolism, immune, gastrointestinal, and cardiovascular activities have been reported for GHSs and ghrelin.[3–6]

Previous studies have revealed that ghrelin administration can improve cardiac function in rats and patients with chronic heart failure, as indicated by increased left ventricle ejection fraction (LVEF), cardiac output, and exercise capacity.[7–9] In rodents with acute myocardial infarction (MI), ghrelin administration prevented malignant arrhythmias and reduced mortality in the acute phase, while improving left ventricle (LV) dysfunction and attenuating cardiac remodeling in the subacute phase.[10–13] However, ghrelin is an unstable natural peptide that is transformed and degraded, which limits its clinical use. The GHS hexarelin is a chemically stable and potent synthetic hexapeptide that can be administered orally, making it a potential alternative to ghrelin.[14] It is comparable to ghrelin with respect to the half-maximal effective concentration for their common receptor, GHSR 1a; although the cardiac action of hexarelin was reported to be mediated in part by GHSR 1a and largely by activation of the CD36 receptor, in isolated working hearts.[15,16] In this concise review, we discuss the current evidence for the cardiovascular action of hexarelin.

2 Cardiovascular action

2.1 Inotropic effect

Acute intravenous administration of hexarelin had a short-lasting, positive inotropic effect. Cardiac performance was studied by radionuclide angiocardiography in seven male volunteers. Hexarelin administration increased LVEF ($70.7 \pm 3.0\%$ *vs.* $64.0 \pm 1.5\%$, $P < 0.03$) without affecting mean blood pressure and heart rate. LVEF was significantly increased after 15 min and peaked at 30 min, and the effect lasted for up to 60 min after administration.[17] In 24 male patients with coronary artery disease undergoing by-pass surgery under general anesthesia, LVEF, cardiac output, and cardiac index were evaluated by transoesophageal echocardiography while wedge pressure, central venous

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pressure, mean arterial pressure, and systemic vascular resistance index were determined by systemic and pulmonary arterial catheterization. Acute intravenous administration of hexarelin (2 μg/kg) induced a rapid increase in LVEF, cardiac output, and cardiac index, while reducing wedge pressure. It also increased mean arterial pressure and transiently decreased central venous pressure, but did not change the systemic vascular resistance index and heart rate.[18] Furthermore, hexarelin induced time- and concentration-dependent inotropic effects in rat papillary muscle,[19] and increased the amplitude of intracellular Ca^{2+} transients and L-type Ca^{2+} current to produce positive inotropic effects in freshly isolated adult Wistar rat ventricular myocytes through protein kinase C signaling cascade.[20].

2.2 Inhibition of apoptosis

Treatment of neonatal rat cardiomyocytes with hexarelin significantly decreased angiotensin II-induced apoptosis and DNA fragmentation, and increased myocyte viability.[21] Hexarelin treatment also inhibited doxorubicin-induced apoptosis and promoted survival of H9c2 cardiomyocytes and endothelial cells.[22] The anti-apoptosis activity of hexarelin in cardiomyocytes and endothelial cells may partially explain its cardioprotective effects. Chronic administration of hexarelin alleviates LV dysfunction, pathological remodeling, and cardiac cachexia in rats with congestive heart failure by suppressing stress-induced neurohormonal activation and cardiomyocyte apoptosis.[23]

2.3 Ischemia-reperfusion injury

In hearts subjected to 30 min of ischemia followed by 120 min of reperfusion, hexarelin (1 μmol/L) significantly reduced infarct size, as determined by using triphenyltetrazolium chloride staining, and the protection provided by hexarelin was partly abolished by the protein kinase C inhibitor chelerythrine.[24] Hexarelin treatment not only preserved the electrophysiological properties of cardiomyocytes after ischemia-reperfusion injury but also inhibited cardiomyocyte apoptosis and promoted cell survival by modification of mitogen-activated protein kinase pathways,[25] and produced a positive inotropic effect on ischemic cardiomyocytes.[26] Hexarelin administration for 30 days counteracted the ischemic heart damage in Zucker rats subjected to low flow ischemia and reperfusion. The recovery of LV pressure developed at reperfusion was significantly greater in hexarelin-treated rats than in controls and the increase in coronary resistance was minimal.[27] The chronic administration of hexarelin to GH-deficient rats had a pronounced protective effect against ischemic and post-ischemic ventricular dysfunction, and prevented hyper-responsiveness of the coronary vascular bed to angiotensin II in perfused hearts.[28]

2.4 Myocardial infarction

Four weeks after ligation of the left coronary artery, male rats were treated with hexarelin (100 μg/kg per day) or normal saline subcutaneously for two weeks. Transthoracic echocardiography was performed before and after the treatment period. Compared with normal saline, hexarelin treatment increased stroke volume, stroke volume index, cardiac output, and cardiac index, and decreased total peripheral resistance.[29]

2.5 Cardiac fibrosis

Hexarelin treatment of spontaneously hypertensive rats for five weeks significantly reduced cardiac fibrosis by decreasing interstitial and perivascular myocardial collagen deposition and myocardial hydroxyproline content, and reducing collagen I and III mRNA and protein expression. In addition, hexarelin treatment increased matrix metalloproteinase-2 and -9 activities and decreased myocardial mRNA expression of the tissue inhibitor of metalloproteinase-1. Furthermore, hexarelin treatment significantly attenuated LV hypertrophy, LV diastolic dysfunction, and high blood pressure.[30] Treatment of cultured cardiac fibroblasts with hexarelin (0.1 μmol/L) inhibited angiotensin II-induced proliferation and collagen synthesis, and transforming growth factor (TGF)-β-induced DNA synthesis, and reduced the angiotensin II-mediated upregulation of TGF-β expression and release.[31]

2.6 Atherosclerosis

Anti-atherosclerotic activity of hexarelin was observed in adult Sprague-Dawley rats. Treatment with hexarelin suppressed the formation of atherosclerotic plaques and neointima, partially reversed serum high-density lipoprotein cholesterol/low-density lipoprotein cholesterol ratio, and increased serum nitric oxide levels and aortic mRNA expression of endothelial nitric oxide synthase, GHSRs, and CD36 in atherosclerotic rats. Hexarelin treatment also decreased tritiated thymidine incorporation in cultured vascular smooth muscle cells, calcium sedimentation in the aortic wall, and foam cell formation induced by oxidized low-density lipoprotein.[32] Furthermore, chronic treatment with hexarelin unaltered the high triglyceride levels and significantly decreased plasma cholesterol concentrations in obese rats.[27]

3 Cardiac receptor

The cardiovascular action of hexarelin has been regarded

as GH-independent and occurs through activation of cardiac receptors. Previous studies showed that the cardiovascular effects of hexarelin are not shared by recombinant human GH or by GH-releasing hormone, indicating that they are not mediated by an increase in circulating GH levels.[17,18,33] Moreover, hexarelin significantly increased LVEF in normal and in GH-deficient patients[34–36] and prevented cardiac damage after ischemia-reperfusion in hypophysectomized rats,[37] indicating that its cardioprotective activity is not due to stimulation of the GH axis.[27]

Hexarelin can bind to specific cardiac sites. Specific ^{125}I-Tyr-Ala-hexarelin binding was observed in the human cardiovascular system, and the highest ^{125}I-Tyr-Ala-hexarelin levels were detected in the ventricles, followed by atria, aorta, coronaries, carotid, endocardium, and vena cava.[38] Specific hexarelin binding has also been shown in H9c2 myocytes.[39] Currently, two cardiac receptor subtypes have been proposed for hexarelin.

3.1 Cardiac GHSR 1a receptor

GHSR mRNA expression in cardiomyocytes was upregulated after treatment with hexarelin,[21] and GHSR 1a protein was expressed primarily in the heart as compared to all other organs.[40] Fluorescein-conjugated ghrelin (1–18) bound specifically to heart tissue *in situ* and was displaced by both excess ghrelin and hexarelin.[40] Further, hexarelin significantly prolonged action potential duration, produced positive inotropic effects, and preserved electrophysiological properties after ischemia-reperfusion injury in isolated myocytes. These effects were abolished in the presence of the GHSR antagonist d-Lys-3-GH-releasing peptide-6 or the GHSR 1a-specific antagonist BIM28163.[20,25,26,41] The effects of hexarelin on cardiac function, cardiac fibrosis, and blood pressure were also mediated by GHSRs, since GHSR expression was upregulated by hexarelin treatment and a selective GHSR antagonist inhibited hexarelin activity.[30]

3.2 Cardiac CD36 receptor

The presence of specific GHS binding sites was demonstrated in three different human breast carcinoma cell lines (MCF7, T47D, and MDA-MB-231), which lacked detectable GHSR 1a mRNA expression. However, hexarelin treatment significantly inhibited proliferation of these cell lines at concentrations close to the binding affinity.[42] A photoactivatable derivative of hexarelin was developed to label and characterize binding sites in anterior pituitary membranes. The differential binding affinity for cardiac tissue raised the possibility of the existence of distinct receptor subtypes in the pituitary and the cardiovascular system.[43] GHSRs were detected mainly in the myocardium by using a radioreceptor assay with ^{125}I-Tyr-Ala-hexarelin, but they were also present in the adrenals, gonads, arteries, lungs, liver, skeletal muscle, kidneys, pituitary, thyroid, adipose tissue, veins, uterus, skin, and lymph nodes. Hexarelin and human ghrelin completely displaced the radioligand from binding sites in endocrine tissues, but ghrelin was less potent than hexarelin. In non-endocrine tissues, such as heart, ghrelin did not displace ^{125}I-Tyr-Ala-hexarelin, whereas hexarelin had the same displacement activity as in endocrine tissues. This suggested that there is a hexarelin-specific receptor subtype in the heart and in other non-endocrine tissues.[44] Finally, the specific cardiac receptor for hexarelin was identified. The N-terminal sequence of the deglycosylated protein was identical to rat CD36, a multifunctional glycoprotein, which is expressed in cardiomyocytes and microvascular endothelial cells. Hexarelin-mediated activation of CD36 in perfused hearts increased coronary perfusion pressure in a dose-dependent manner. This effect was not observed in hearts from CD36-null mice and from spontaneously hypertensive rats genetically deficient in CD36.[45, 46]

4 Hexarelin *vs*. ghrelin

Hexarelin has more potent beneficial effects on the cardiovascular system compared with its natural analog ghrelin. In one study, either ghrelin (320 μg/kg per day) or equimolar hexarelin (80 μg/kg per day) was administered to hypophysectomized rats for seven days and their hearts were then subjected to ischemia and reperfusion *in vitro*. Hexarelin was more potent than ghrelin in preventing increases in LV end-diastolic pressure, coronary perfusion pressure, and creatine kinase release in the heart perfusate.[15] In another study, chronic hexarelin administration improved heart function in ghrelin-null mice to a greater extent than equimolar ghrelin administration after experimental MI.[47] Given the fact that the half-maximal effective concentration of hexarelin for GHSR 1a (1.7 nmol/L) is comparable to that of ghrelin (1.0 nmol/L),[16] the higher potency of hexarelin was considered to be mediated largely by interactions with CD36 in the heart, and in part by GHSRs.[15,47]

However, other studies reported that when GHSR 1a activation was identical hexarelin and ghrelin had similar cardiac effects, although the dosage of ghrelin was 10 times higher than that of hexarelin in molar terms. Ghrelin (10 nmol/L) or hexarelin (1 nmol/L) addition to the perfusion system after ischemia had a positive inotropic effect on ischemic cardiomyocytes through activation of the GHSR 1a receptor, thereby protecting them from ischemia-reperfusion injury.[20,26] Another study suggested that ghrelin-

and hexarelin-mediated activation of GHSR 1a had a similar protective effect on cardiomyocytes after ischemia-reperfusion injury by inhibiting cardiomyocyte apoptosis and promoting cell survival.[25] The common features of the two peptides were compared in Table 1.

5 Conclusions

Hexarelin has cardioprotective activity in common cardiovascular conditions such as cardiac fibrosis, ischemic heart disease, cardiac dysfunction, and atherosclerosis. The important *in vivo* studies of hexarelin in cardiovascular conditions are summarized in Table 2. These beneficial effects seem to be mediated through the direct binding and activation of its cardiac receptors CD36 and GHSR 1a. Since hexarelin is a chemically stable synthetic GHS with more potent cardiac effects than its natural analog ghrelin, it can be a potential alternative to ghrelin as a promising

Table 1. Comparison of hexarelin and ghrelin.

	Hexarelin	Ghrelin
Source	Synthetic	Natural
Chemical structure	6 Amino acids	28 Amino acids
Amino acid sequence	His-D-2-methyl-Trp-Ala-Trp-D-Phe-Lys-NH2	Gly-Ser-Ser(octanoyl)-Phe-Leu-Ser-Pro-Glu-His-Gln-Arg-Val-Gln-Gln-Arg-Lys-Glu-Ser-Lys- Lys-Pro-Pro-Ala-Lys-Leu-Gln-Pro-Arg-OH
Half life	57–71 min[48]	11–17 min,[49] 27–31 min[50]
Receptor	GHSR1a; CD36	GHSR1a
Receptor affinity for GHSR1a (EC50)	1.7 nmol/L[16]	1.0 nmol/L[16]

GHSR1a: growth hormone secretagogue receptor; EC50: half-maximal effective concentration.

Table 2. *In vivo* studies of the cardiovascular action of hexarelin.

First author, date	Species	Model	Dose, duration, initiation of treatment	Main outcomes
Mao, *et al*, 2013[47]	Ghrelin-null mice	Experimental myocardial infarction by coronary artery ligation	300μg/kg per day for 14 days, from 30 min after ligation (*s.c.*)	Improved heart failure better than ghrelin
Xu, *et al*, 2012[30]	Rats	Spontaneous hypertension	100 μg/kg per day for 5 weeks, from an age of 16 weeks (*s.c.*)	Reduced cardiac fibrosis
Pang, *et al*, 2010[32]	Rats	High lipid diet and vitamin D3-induced atherosclerosis	200 μg/kg per day for 30 days, in the last month after high lipid diet (*s.c.*)	Alleviated the development of atherosclerosis
Xu, *et al*, 2005 [23]	Rats	Pressure-overload heart failure by abdominal aortic banding	200 μg/kg per day for 3 weeks, from 9 weeks after heart failure (*s.c.*)	Alleviated LV dysfunction, pathological remodeling, and cardiac cachexia
Torsello, *et al*, 2003 [15]	Rats	Hypophysectomized	80 μg/kg per day for 7 days, before *in vitro* ischemia and reperfusion procedure (*s.c.*)	Far more effective than ghrelin in the control of heart function
Broglio, *et al*, 2002 [18]	Humans	Coronary artery disease during by-pass surgery	2 μg/kg acute administration (*i.v.*)	Increased LVEF, cardiac index and cardiac output
Imazio, *et al*, 2002 [34]	Humans	Normal, dilated, and ischemic cardiomyopathy	2 μg/kg acute administration (*i.v.*)	Increased LVEF in ischemic cardiomyopathy patients and in normals but not in dilated cardiomyopathy patients
Broglio, *et al*, 2001 [35]	Humans	Normal adults, growth hormone-deficient patients, and severe dilated cardiomyopathy patients	2 μg/kg acute administration (*i.v.*)	Produced a positive inotropic effect
De Gennaro-Colonna, *et al*, 2000 [27]	Zucker rats	Obese	160 μg/kg per day for 30 days, at 30 weeks of age (*s.c.*)	Induced cardioprotective effect after ischemia and decreased plasma cholesterol
Tivesten, *et al*, 2000 [29]	Rats	Experimental myocardial infarction by coronary artery ligation	10 μg/kg per day or 100μg/kg per day for 2 weeks, from 4 weeks after ligation (*s.c.*)	Improved cardiac function and decreased peripheral resistance
Bisi, *et al*, 1999[36]	Humans	Growth hormone deficiency	2μg/kg acute administration (*i.v.*)	Increased LVEF
Locatelli, *et al*, 1999[37]	Rats	Hypophysectomized	80 μg/kg per day for 7 days, before ischemia-reperfusion damage (*s.c.*)	Prevented cardiac damage after ischemia-reperfusion
Bisi, *et al*, 1999[17]	Humans	Volunteers	2μg/kg acute administration (*i.v.*)	Increased LVEF without significant changes in mean blood pressure and heart rate
De Gennaro Colonna, *et al*, 1997[51]	Rats	Anti-GHRH serum-treated	160 μg/kg per day for 15 days, after administration of an anti-GHRH serum for 20 days (*s.c.*)	Counteracted the ischemic damage

LV: left ventricle; LVEF: left ventricular ejection fraction; GHRH: growth hormone releasing hormone.

therapeutic agent for the treatment of cardiovascular diseases. However, as current evidence is mainly from experimental animal models or *in vitro* cell lines, clinical trials aimed to extend the application of hexarelin in human subjects and observe its efficacy and potential side effects are warranted.

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