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Research Paper

One dose of oral hexarelin protects chronic cardiac function after myocardial infarction

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ABSTRACT

Both hexarelin and its natural analog ghrelin exert comparable cardioprotective activities. A single dose of ghrelin administered at the very acute phase after experimental myocardial infarction positively affects cardiac function in chronic heart failure. Therefore, this study aimed to determine whether a single dose of oral hexarelin has the same effect in the chronic disease phase. Myocardial infarction or sham operation was generated by left coronary artery ligation in male C57BL/6J mice, which subsequently received one dose of hexarelin or vehicle treatment by oral gavage 30 min after operation. Although the mortality within 14 days after myocardial infarction did not differ between the groups, hexarelin treatment protected cardiac function in the chronic phase as evidenced by higher ejection fraction and fractional shortening, as well as lower lung weight/body weight and lung weight/tibial length ratios, compared with vehicle treatment. Hexarelin treatment concurrently lowered plasma epinephrine and dopamine levels, and shifted the balance of autonomic nervous activity toward parasympathetic nervous activity as evidenced by a smaller low/high-frequency power ratio and larger normalized high-frequency power on heart rate variability analysis. The results first demonstrate that one dose of oral hexarelin treatment potentially protects chronic cardiac function after acute myocardial infarction, and implicate that activating growth hormone secretagogue receptor 1a might be beneficial for cardioprotection, although other mechanism may also be involved.

Introduction

Acute myocardial infarction (MI) is one of the leading causes of death and heart failure worldwide; it is the single largest cause of death in the United States, where it is responsible for one-sixth of all deaths [28,40]. Among elderly patients who survived their first acute MI, 76% develop heart failure within 5 years [7]. Despite rapid advancements in pharmacological and surgical approaches over the last several decades, heart failure after acute MI remains a serious problem.

Ghrelin, a growth hormone-releasing peptide mostly produced from the stomach and small bowels, is an endogenous ligand for growth hormone secretagogue receptors (GHSRs) [13]. Several studies demonstrate the beneficial effects of exogenous administration of ghrelin after acute MI, such as preventing malignant arrhythmias and reducing mortality in the acute phase, whereas continuous administration improving left ventricle (LV) dysfunction and attenuating cardiac remodeling in the chronic phase [30,31]. Furthermore, ghrelin-knockout mice exhibit an increased incidence of malignant arrhythmias in the very acute phase and deteriorated heart function and excessive sympathoactivation in the chronic phase after MI [20,21]. A recent study demonstrates a single dose of ghrelin administered subcutaneously to rats 30 min after experimental MI prevents elevated in cardiac sympathetic nervous activity (SNA) and improves early survival prognosis; these early beneficial effects of ghrelin can also positively affect cardiac

Abbreviations: BW, body weight; %FS, percentage fractional shortening; GHSs, growth hormone secretagogues; GHSRs, growth hormone secretagogue receptors; HW, heart weight; LF/HF, the ratio of LF to HF power; LV, left ventricle; LVAWth, LV anterior wall thickness; LVDd, LV end-diastolic diameter; LVDs, LV end-systolic diameter; LVPWth, LV posterior wall thickness; LW, lung weight; MI, myocardial infarction; nHF, normalized HF power; nLF, normalized LF power; slope-EDPVR, slope of the end-diastolic pressure–volume relationship; slope-ESPVR, slope of the end-systolic pressure–volume relationship; SNA, sympathetic nervous activity; TL, tibial length.

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function in chronic heart failure [29]. These findings imply that ghrelin injection may be a potential clinical treatment for acute MI to protect chronic cardiac function via modulation of cardiac SNA. However, ghrelin is an unstable natural peptide that is transformed and degraded, limiting its clinical use.

Growth hormone secretagogues (GHSs) are a heterogeneous group of synthetic peptides and nonpeptides; as exogenous ligands, they can bind GHSRs and stimulate growth hormone secretion in both animals and human subjects. Among GHSs, hexarelin is a stable and effective GHS that can be administered orally, making it a potential alternative to ghrelin [6]. It is comparable to ghrelin with respect to the half-maximal effective concentration of their common receptor, GHSR 1a, although the cardiac action of hexarelin was reported to be mediated in part by the GHSR 1a and largely by activation of receptor CD36 on isolated working hearts [8,37]. Therefore, this study aimed to clarify whether a single dose of oral hexarelin treatment at the very acute phase after MI has protective effects on chronic cardiac function similar to ghrelin.

Material and methods

Animal models of MI

All experiments were approved by the animal ethics committee of the National Cerebral and Cardiovascular Center Research Institute and conducted in accordance with the guidelines of the Physiological Society of Japan.

MI was induced in male mice (background strain: C57BL/6J; 14–16 weeks of age) as follows: all the animals received a subcutaneous injection of carprofen analgesia (5 mg/kg) and strepcin antibiotics (0.1 mL/kg) prior to surgery. They were subsequently anesthetized with isoflurane (induced at 4% and maintained at 1.5%), intubated, and supported by an animal ventilator (stroke volume, 150 μL; respiratory rate, 135 strokes/min). After left thoracotomy, the left anterior descending coronary artery was ligated 3 mm from its origin using a 6-0 Prolene suture. Sham-operated mice underwent the same surgical procedure without coronary artery ligation. The chest was closed, and the mice remained on a heating blanket during recovery from anesthesia. The mice were then returned to their standard housing conditions, where they remained for 14 days before experimental data were collected. During this period, the welfare of the animals was monitored by recording body weight (BW), food and water consumption, observing general appearance and behavior, and cleaning and dressing wounds if required. The mice were housed in a 12-h light/12-h dark cycle at 25 °C and provided food and water ad libitum.

Hexarelin and vehicle administration

Hexarelin (600 μg per mouse) or vehicle was administered to 24 mice each by oral gavage 30 min after the MI procedure (hexarelin- or vehicle-treated group), and 10 mice after sham operation were also received oral vehicle administration (sham-operated group). The dose of oral hexarelin was chosen to be as effective as 40 μg/kg subcutaneous administration [10], which is approximately equimolar with that of ghrelin in the previous study [29].

Echocardiographic examination

Echocardiography was performed using an echocardiographic system equipped with an 18-MHz phased-array transducer (MS400, VisualSonics Inc., Ontario, Canada) under anesthesia (1.5% isoflurane) on day 14 after the experimental MI or sham operation. LV end-diastolic diameter (LVDd), LV end-systolic diameter (LVDs), percentage fractional shortening (%FS), LV anterior wall thickness (LVAWth), and LV posterior wall thickness (LVPWth) were calculated.

Heart rate variability analysis

The day after echocardiography, electrocardiogram signals were recorded using a physiological analyzing system (Bio Amp, AD Instruments, CA, USA). Mice were anesthetized with intraperitoneal injections of combination urethane-chloralose (750 and 35 mg/kg, respectively) with supplemental doses as needed [1]. After the heart rate stabilized, electrocardiogram signals were recorded for a minimum of 15 min. Autonomic nervous function was examined by a power spectral analysis of heart rate variability (LabChart Pro 7.0, ADInstruments, Sydney, Australia). The heart rate data were fast Fourier-transformed and used to generate a power spectral density curve. The area under the curve was calculated for the very-low-frequency (VLF: 0.1–0.4 Hz), low-frequency (LF: 0.4–1.5 Hz), and high-frequency (HF: 1.5–5.0 Hz) bands, as described previously [35]. These data were used to calculate the parameters of normalized LF power (nLF), normalized HF power (nHF), and the ratio of LF to HF power (LF/HF).

Arterial blood pressure and cardiac function measurement

After heart rate recording, hemodynamics studies were also performed under anesthesia as described previously [27]. Briefly, after the mice were intubated and connected to a ventilator, a 1F Mikro-Tip Ultra-Miniature Pressure–Volume catheter (Millar Instruments, Houston, TX, USA) was inserted into the LV via the right carotid artery to record baseline hemodynamics in the closed chest using the Pressure–Volume Conductance System (Millar Instruments) connected to a physiological recorder (PowerLab System, AD Instruments, Mountain View, CA, USA). A right thoracotomy was subsequently performed, followed by transient occlusion of the thoracic vena cava by a silk thread placed around it immediately above the diaphragm; this was performed to vary the venous return during the recording of hemodynamics. Parallel conductance (Vp) was subsequently determined by a 10-μL injection of 15% saline into the left jugular vein to establish the offset due to the conductivity of structures external to the blood pool. The derived Vp was used to correct the pressure–volume loop data. All the data were analyzed using the PVAN 3.4 software package (Millar Instruments).

Histological examination

At the end of the hemodynamics studies, the animals were euthanized by bilateral pneumothoracotomy. The hearts were bisected from the apex to the base into 2 equal transverse sections. The apex was fixed with 4% paraformaldehyde and embedded in paraffin. Paraffin sections were prepared with Masson's trichrome, and hematoxylin and eosin staining to measure infarct size. The sections were subsequently mounted and photographed. The total infarct size was determined by measuring the infarct area of each section, multiplying the area by the slice thickness, and summing the results from all slices. Infarct sizes are presented as the percentage of the total LV wall.

Catecholamine measurement

Blood samples were collected into heparinized tubes from the mice 14 days after MI or sham operation. The plasma was separated by centrifugation (4 °C, 3000 rpm for 30 min) immediately and stored at −80 °C until it was assayed. The plasma concentrations