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Cardioprotective effects of ghrelin and des-octanoyl ghrelin on myocardial injury induced by isoproterenol in rats[1]

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Key words

ghrelin; des-octanoyl ghrelin; myocardium fibrosis; isoproterenol

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Abstract

Aim: To determine the cardioprotective action of ghrelin and des-octanoyl ghrelin in rats with isoproterenol-induced myocardial injury. **Methods:** Rats were subcutaneously injected with isoproterenol (ISO; 20, 10, and 5 mg/kg) on d 1, 2 and 3, respectively, and then 3 mg/kg for the next 7 d with or without ghrelin or des-octanoyl-ghrelin (100 μg/kg, twice daily). Plasma ghrelin and growth hormone levels were assayed using radioimmunoassay methods. Growth hormone secretagogue receptor (GHSR) and ghrelin mRNA were determined using RT-PCR. The maximal binding capacity and the affinity for [^{3}H]ghrelin were determined by receptor binding assays. **Results:** Compared with controls, ISO-treated rats showed severe myocardial injury, cardiomegaly, infarction-like necrosis and massive fibrosis with increases in irradiated-ghrelin (ir-ghrelin) content in plasma by 67% and myocardia by 66% and in the mRNA level in the myocardia by 93% (P<0.01). ISO-treated rats had 95% (P<0.01) higher GHSR mRNA levels in the myocardia. The maximal binding capacity of [^{3}H]ghrelin for myocardial sarcolemma was higher in ISO-treated rats than in controls. Ghrelin administration improved cardiac function and ameliorated cardiomegaly and attenuated myocardial lipid peroxidation injury and relieved cardiac fibrosis as compared with ISO treatment alone. Administration of des-octanoyl ghrelin effectively antagonized ISO-induced myocardial injury and improved all parameters measured. However, the therapeutic effect of des-octanoyl ghrelin was significantly weaker than that of ghrelin. The plasma growth hormone level increased markedly, by 1.5-fold (P<0.01), with ghrelin administration as compared with that in controls, but was unaltered in the des-octanoyl ghrelin group. **Conclusion:** Myocardial ghrelin and GHSR were up-regulated during ISO-induced myocardial injury. The protective effect of ghrelin against ISO-induced cardiac function injury and fibrosis was more potent than that of des-octanoyl ghrelin, which suggests that ghrelin could be an endogenous cardioprotective factor in ischemic heart disease, and that its effects include growth hormone-dependent and -independent pathways.

Introduction

Ghrelin, a novel 28-amino acid peptide principally released from the stomach, has been identified as an endogenous ligand for the growth hormone secretagogue receptor (GHSR)[1]. GHSR-1a, a G protein-coupled receptor mainly expressed in the pituitary and hypothalamus, is thought to mediate the endocrine actions of ghrelin, including its potent growth hormone (GH)-secreting effects[2]. Ghrelin mainly circulates in 2 different forms: octanoyl and des-octanoyl ghrelin.

Although ghrelin is essentially a gastro-derived hormone, it has been found to be expressed ubiquitously and circulated in plasma at a concentration of approximately 100 pmol/L in healthy humans[3]. Recently, growing evidence has indicated that cardiovascular tissue is rich in ghrelin receptors, and that its mRNA is expressed abundantly in human myocardial cells, and rat ventricles, atria, aorta, coronary arteries, carotid arteries, the endocardium and vena cava[4], which confirms that the cardiovascular system is a target for ghrelin. In addition, ghrelin is an important autocrine/paracrine factor of cardiovascular tissues and has various cardiovascular effects, including increased myocardial contractility and vasodilatation, and anti-inflammation[5,6]. Our previous work showed that ghrelin was an endogenous cardioprotective factor in isolated rat hearts with ischemia/reperfusion injury, ghrelin perfusion enhanced coronary flow, ameliorated cardiac function suppression and decreased myocardial oxygen consumption[7]; in rats with heart failure induced by sepsis, ghrelin improved cardiac function and hemodynamics[8]; and in rats with acute myocardial necrosis induced by isoproterenol (ISO; 40 mg/kg per d, for 2 d) ghrelin administration significantly protected the heart from ischemic injury and lipid peroxidation damage[9]. However, the cardioprotective mechanism of ghrelin is still unclear.

From past studies, the effect of ghrelin was thought to depend entirely on its acylation and subsequent interaction with GHSR to promote the synthesis and secretion of GH. Recent investigations have revealed that besides having a stimulatory effect on GH secretion, ghrelin possesses a variety of effects through GH-independent mechanisms. Tsubota and coworkers reported that the microinjection of ghrelin into rat nucleus tractus solitarii (NTS) elicited hypotensive effects, but this was not the case upon injection into GHSR-expressing rostral ventrolateral medulla or caudal ventrolateral medulla. To elucidate the mechanism of ghrelin, they examined the cardiovascular response of rats microinjected with des-acyl ghrelin into NTS. Des-acyl ghrelin does not activate GHSR-1a and is devoid of endocrine activity. The hypotensive and bradycardic activity evoked by des-acyl ghrelin was not significantly different from that of native ghrelin. These results suggest that des-acyl ghrelin contributes to the regulation of cardiovascular control and that a receptor other than GHSR-1a exists in NTS. In addition, several reports have shown that ghrelin plays many roles that are independent of GH release[10–12]. However, it is unknown whether the cardioprotective role of ghrelin is absolutely or partly dependent on GH release.

In the present study, we used an ISO-induced myocardial injury model to observe the changes in expression of cardiac ghrelin and its receptor, and to investigate the therapeutic effects of ghrelin and des-octanoyl ghrelin and the mechanisms thereof.

Materials and methods

Animals and reagents All animal care and experimental protocols were complied with the Animal Management Rules of the Ministry of Health of the People's Republic of China (document No 55, 2001) and the guidelines for the Care and Use of Laboratory Animals of the First Hospital, Peking University. Male Sprague-Dawley (SD) rats (250–300 g) were provided by the Animal Department of the Health Science Center of Peking University. All animals were maintained on normal rat chow, had free access to water, and were kept in conditions of 12 h light/12 h dark cycle. Synthetic human ghrelin, des-octanoyl ghrelin, [^{3}H]ghrelin (54 mCi/mmol) and radioimmunoassay kits for rat ghrelin were provided by Phoenix Pharmaceuticals (St Joseph, MO, USA). The radioimmunoassay kit for rat GH was provided by Eiken Chemicals (Tokyo, Japan). ISO and aprotinin were purchased from Sigma (St Louis, MO, USA), and the kit for hydroxyproline was from Jiancheng Biotechnology Institute (Nanjing, China). Trizol was from Gibco (Rockville, MD, USA), and dNTP, MuLV reverse transcriptase, Oligo(dT)$_{15}$, and *Taq* DNA polymerase were from Promega (Madison, WI, USA). Oligonucleotide primer sequences were as follows: ghrelin-S, 5'-CTT CTT GAG CCC AGA GCA C-3' and ghrelin-A, 5'-GTG GCT GCA GTT TAG CTG GT-3', used for the amplification of ghrelin cDNA; GHSR-S, 5'-CTA TCC AGC ATG GCC TTC TC-3' and GHSR-A, 5'-GGA AGC AGA TGG CGA AGT AG-3', used for the amplification of GHSR cDNA; and beta-actin-S, 5'-ATC TGG ACC ACC TTC-3', and beta-actin-A, 5'-AGC CAG GTC CAG ACG CA-3', used for the amplification of β-actin for calibrating sample loading. All sequences of oligonucleotide primers were synthesized by Sai Bai Sheng (Beijing, China). Other chemicals and reagents were of analytical grade.

Preparation of animal model The ISO-induced subacute myocardial injury model was produced as described previously[13], with minor modifications. Thirty male SD rats were randomly and equally divided into 5 groups: (1) ISO group: ISO (20, 10, and 5 mg/kg) was subcutaneously injected on d 1, 2, and 3, respectively, and then 3 mg/kg for the next 7 d; (2) control group: rats were subcutaneously injected with normal saline for 10 d; (3) and (4) therapeutic groups: ISO administration was the same as in (1), and ghrelin and des-octanoyl ghrelin (100 μg/kg, twice daily), respectively, were subcutaneously injected for 10 d.

At the end of the experiment all rats were fasted overnight but had free access to water at the last administration of the drug. The rats were anesthetized with urethane (1 g/kg, ip), and a catheter filled with heparin saline (500 U/mL) was inserted into the right common carotid artery for measuring intraventricular pressure. The heart rate, maximal left-ventricular pressure development (LV dp/dt_{max}) and left ventricular end-diastolic pressure (LVEDP) were recorded by using Powerlab/4S (Santa Monica, CA, USA). After the hemodynamic parameters were measured, a blood sample was drawn from the left ventricle and mixed with 1 mg/mL ethylenediamine tetraacetic acid (EDTA)-2Na and 500 kIU/mL aprotinin. Serum was obtained by centrifugation at 600×*g* for 10 min at 4 ºC and stored at -70 ºC. Hearts were removed and weighed. The heart apex was taken for pathological examination under hematoxylin-eosin staining. The left ventricular (LV) myocardial sample (100 mg) was acidified by adding 1 mmol/L acetic acid, boiling for 10 min and homogenizing. The tissue supernatant was stored at -70 ºC and used for ghrelin radioimmunoassay.

Plasma lactate dehydrogenase (LDH) activity was measured on an automatic biochemistry analyzer. The content of lipid peroxidation product malondialdehyde (MDA) and conjugated diene (CD) in myocardia and plasma was determined by using the thiobarbituric acid test[14] and spectrometry[15], respectively, in duplication. The collagen content in the LV myocardium was determined by hydroxyproline assay as described previously[16].

Assay of mRNA levels of ghrelin and GHSR by RT-PCR[17] Total RNA from the LV myocardium (approximately 50 mg) was extracted by using Trizol reagent. A total of 1 μg of total RNA was reverse-transcribed into single-strand cDNA with oligo(dT) 15 primer and M-MuLV reverse transcriptase. RT-PCR was performed in a 0.2 mL tube containing 2 μL tissue cDNA, 1 μL of 5 μmol/L solutions of each S and A primer, 1 μL of 2.5 mmol/L of each dNTP mixture, 1.5 μL of 1.5 mmol/L $MgCl_2$, 2.5 μL 10× PCR buffer, and 1.25 U *Taq* DNA polymerase, in a total volume of 25 μL. RT-PCR of ghrelin was as follows: after being denatured at 95 ºC for 5 min, the solution underwent PCR at 94 ºC for 30 s, 55 ºC for 30 s, and 72 ºC for 40 s for 30 cycles. RT-PCR of GHSR was as follows: after being denatured at 95 ºC for 5 min, the solution underwent PCR at 94 ºC for 30 s, 58 ºC for 30 s, and 72 ºC for 40 s for 30 cycles. The 6 μL PCR product was separated on a 1.5% agarose gel and stained with ethidium bromide. The optical density of the band of ghrelin mRNA (374 bp) and GHSR mRNA (321 bp) was measured by use of the Gel Documentation System (Bio-Rad, Hercules, CA, USA). The PCR products were amplified again at 94 ºC for 30 s, 55 ºC for 30 s, and 72 ºC for 30 s for 20 cycles with the rat β-actin primers β-actin-S and β-actin-A. The optical density of the β-actin mRNA band (291 bp) was measured. The ratio of ghrelin and GHSR mRNA to β-actin mRNA was considered the relative amount of ghrelin and GHSR mRNA, respectively.

Radioimmunoassay of ghrelin and GH Plasma and the supernatant extracted from myocardia were loaded onto a Sep-Pak C_{18} cartridge after equilibration with normal saline. The cartridge was washed with 2.5 mL normal saline and 10% acetonitrile in 0.1% trifluoroacetic acid, then eluted with 2 mL 50% acetonitrile in 0.1% trifluoroacetic acid. The elution was lyophilized and subjected to radioimmunoassay for ghrelin and GH determination. The IC_{50} for ghrelin was 6.95 pmol/tube and the reactivity with rat ghrelin was 100%. No cross-reactivity was seen with leptin, orexin A and B, neuropeptide Y, galanin, or vasoactive intestinal polypeptide. The lowest sensitivity of GH was 0.1 ng/mL and the reactivity with rat GH was 100%.

[^{3}H]Ghrelin binding to myocardial sarcolemmal membrane Crude myocardial sarcolemmal membrane was prepared as previously described[18] with minor modifications, and protein content was determined by using the Bradford method[19]. The activity of membrane marker enzymes was measured as described previously by our laboratory[20]. Marker enzyme activity of membrane fractions isolated from control hearts and ISO-treated hearts showed the sarcolemmal membrane Na^+-K^+-ATPase activity (6.50±0.60 mmol/g protein) to be 5.8-fold higher than that of the homogenate of myocardia (0.96±0.10 mmol/g protein).

The ghrelin-receptor binding assay was carried out using [^{3}H]ghrelin as a radioligand. The standard assay mixture contained 10 mmol/L $MgCl_2$, 50 mmol/L Tris-HCl (pH 7.4), and [^{3}H]ghrelin (1–40 nmol/L) in the presence or absence of unlabeled ghrelin (20 μmol/L) in a final volume of 0.2 mL. The assay mixture was preincubated at 37 ºC for 2 min, and the binding assay was initiated by the addition of sarcolemmal membrane (50 μg protein) and allowed to proceed for 60 min at 25 ºC. At the end of incubation, the reaction mixture was diluted with 4 mL ice-cold washing buffer (10 mmol/L $MgCl_2$, 50 mmol/L Tris-HCl, pH 7.4) and filtered immediately on Millipore membranes through 0.45-μm glass-fiber filter paper (Baxter Healthcare, Ireland) under suction. The filter paper was washed 3 times with 5 mL washing buffer (20 mmol/L Tris, 2 mmol/L $MgCl_2$, pH 7.4) and dried, and the radioactivity was determined by using a multi-purpose scintillation counter (LS6500; Beckman, CA, USA). The specific binding was defined as the bound radioactivity displaceable by 20 μmol/L of ghrelin. All binding experiments were performed in duplicate. The maximal binding capacity (B_{max}) and the

affinity [the reciprocal of the dissociation constant (K_d)] for [^{3}H]ghrelin were calculated from Scatchard plot analysis results.

Morphological analysis Hearts were isolated and the cardiac apex was fixed in 10% formalin in phosphate-buffered saline (PBS). Tissue was embedded in paraffin, and transverse sections were cut and stained with hematoxylin and eosin or 0.1% picrosirius red to stain collagen.

Statistical analysis Data are expressed as mean±SD. Comparisons between more than 2 groups were carried out by using analysis of variance (one-way ANOVA), then the Student-Newman-Keuls test. Linear regression analysis was used to assess the correlation between variables. $P<0.05$ was considered statistically significant.

Results

ISO induced myocardial ischemia injury and cardiac fibrosis The hearts of rats treated with ISO alone became markedly enlarged, and the heart weight and the heart coefficient (ratio of heart weight to body weight) were increased by 42% and 58%, respectively (all $P<0.01$) as compared with the control group. Compared with control rats, ISO-treated rats showed lower +LV dp/dt_{max} and -LV dp/dt_{max} values, decreased by 66% and 65%, respectively ($P<0.01$) and higher LVEDP, by 2.4-fold ($P<0.01$; Table 1). LDH activity in plasma was greatly increased, by 3.9-fold ($P<0.01$); the MDA and CD content was elevated in myocardia, by 74% and 69%, respectively ($P<0.01$), and in plasma, by 180% and 52%, respectively ($P<0.01$; Table 2). Histological sections of ISO-treated hearts showed widespread subendocardial necrosis, muscle fibers with capillary dilatation, leukocytic infiltration, swelled fibroblasts (Figure 1A), and abundant fibroblastic hyperplasia (Figure 1B).

Treatment with ISO increased myocardial ghrelin content and mRNA level Compared with the control group, ISO-treated rats showed increased irradiated-ghrelin (ir-ghrelin) content in plasma by 67% (246±17 *vs* 410±32 nmol/L, $P<0.01$) and myocardia by 66% (10.41±0.57 *vs* 17.24+1.38 fmol/mg protein, $P<0.01$), and increased mRNA level in myocardia, by 93% ($P<0.01$; Figure 2). The ir-ghrelin content in myocardia and plasma was negatively correlated with +LV dp/dt_{max} (r=-0.713 and r=-0.672, respectively, both $P<0.01$) and -LV dp/dt_{max} (r=-0.668 and r=-0.584, respectively, both $P<0.01$); and positively correlated with LVEDP (r=0.845, $P<0.01$) and (r=0.864, $P<0.01$, respectively) but not hydroxyproline content (r=0.338 and r=0.284, respectively, both $P>0.2$).

Treatment with ISO increased mRNA level of myocardial GHSR and its binding capacity Compared with the con-

Table 1. Alteration of heart weight, heart weight/body weight, ±LVdp/dt_{max} and LVEDP of rats with heart failure after treatment with isoproterenol and ghrelin. n=6. Data are mean±SD. $^cP<0.01$ *vs* control. $^fP<0.01$ *vs* ISO group. $^iP<0.01$ *vs* ghrelin+ISO.

	Controls	ISO	Ghrelin+ISO	Des-octanoyl ghrelin+ISO
Body weight (kg)	0.31±0.02	0.28±0.03	0.31±0.03	0.28±0.04
Heart weight (g)	1.08±0.08	1.53±0.04^c	1.31±0.03^f	1.40±0.03^{fi}
Heart weight/body weight	3.43±0.13	5.42±0.37^c	4.27±0.15^f	4.95±0.14^{fi}
+LV dp/dt_{max} (mmHg/s)	6022±410	2022±182^c	4846±584^f	3106±150^{fi}
–LV dp/dt_{max} (mmHg/s)	5382±164	1878±105^c	4206±864^f	3198±226^{fi}
LVEDP (mmHg)	8.78±1.86	29.87±2.81^c	19.36±2.17^f	24.33±2.88^{fi}

Table 2. Alteration of plasma LDH activity, and MDA and CD content and myocardial MDA content. n=6. Data are mean±SD. $^cP<0.01$ *vs* control. $^fP<0.01$ *vs* ISO group. $^iP<0.01$ *vs* ghrelin+ISO.

	Controls	ISO	Ghrelin+ISO	Des-octanoyl ghrelin+ISO
Content of MDA in heart (nmol/mg protein)	0.27±0.01	0.48±0.05^c	0.31±0.02^f	0.42±0.03^{fi}
Content of MDA in plasma (nmol/mL)	3.02±0.27	8.48±0.30^c	6.21±0.22^f	6.88±0.32^{fi}
Content of CD in heart (nmol/mg protein)	0.58±0.06	0.98±0.04^c	0.61±0.06^f	0.86±0.08^{fi}
Content of CD in plasma (nmol/mg protein)	0.44±0.03	0.68±0.02^c	0.54±0.03^f	0.56±0.02^f
Content of hydroxyproline in heart (mg/mg protein)	0.21±0.03	0.47±0.04^c	0.28±0.03^f	0.38±0.05^{fi}
Content of LDH in plasma (IU)	46.22±2.87	227.4±16.9^c	182.7±16.4^f	204.7±12.2^{fi}

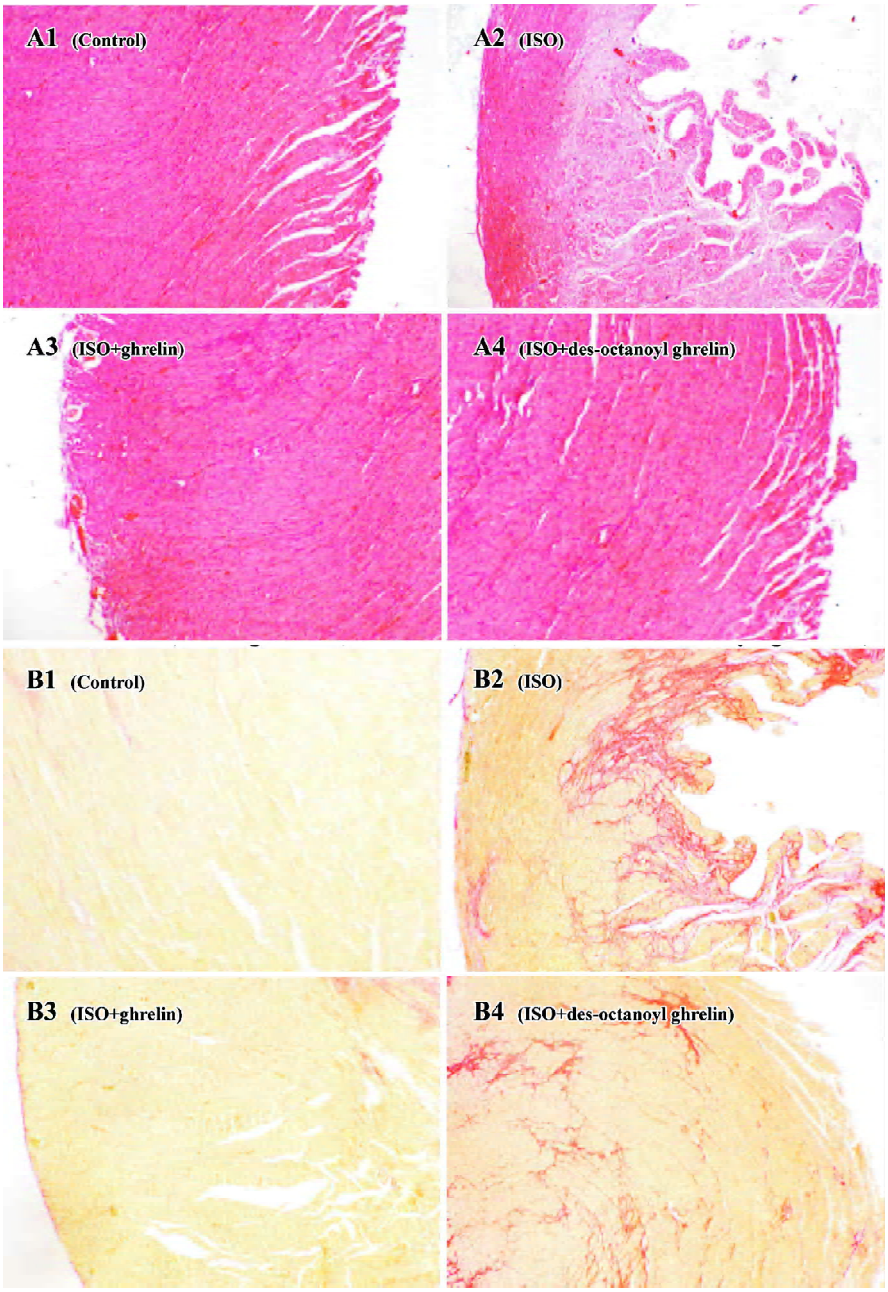

Figure 1. Histological staining results. A1–A4: hematoxylin-eosin staining of myocardia. Myocardial cells of ISO-treated rats were hypertrophic, and were wider than those of the control group; necrotic foci were frequently seen. Ghrelin and des-octanoyl ghrelin treatment ameliorated myocardial hypertrophy and necrosis. B1–B4: picrosirius red-stained left-ventricular sections showing increased interstitial collagen (pink color) in ISO-treated rats compared with control rats. Ghrelin and des-octanoyl ghrelin treatment decreased interstitial collagen markedly. ×200

trol group, ISO-treated rats showed higher GHSR mRNA levels in the myocardia, increased by 95% ($P<0.01$; Figure 2). [^{3}H]Ghrelin binding to cardiac sarcolemmal membranes was found to be a saturable process, with single-component binding being characteristic of all experimental groups. In the sarcolemmal membrane, the B_{max}, calculated from the results of a Scatchard plot (Figure 3), was increased by 87% in the ISO group as compared with controls (34.38±1.66 *vs* 18.30±1.64 fmol/mg protein, $P<0.01$). The affinity (K_d) for [^{3}H]ghrelin binding in sarcolemmal membranes (Figure 3) was not significantly altered (4.31±0.42 *vs* 4.75±0.51 nmol/L, $P>0.05$).

Administration of ghrelin or des-octanoyl ghrelin attenuated ISO-induced myocardial injury Compared with the ISO-alone group, rats given ghrelin showed decreased heart weight and heart coefficient by 14% and 21% (all $P<0.01$), respectively; +LV dp/dt_{max} and -LV dp/dt_{max} were higher, increased by 1.4- and 1.2-fold ($P<0.01$), respectively; and LVEDP was lower, decreased by 34% ($P<0.01$, Table 1). Myocyte necrosis, as indicated by myocardial LDH leakage, was ameliorated ($P<0.01$); and lipid peroxidation product, MDA

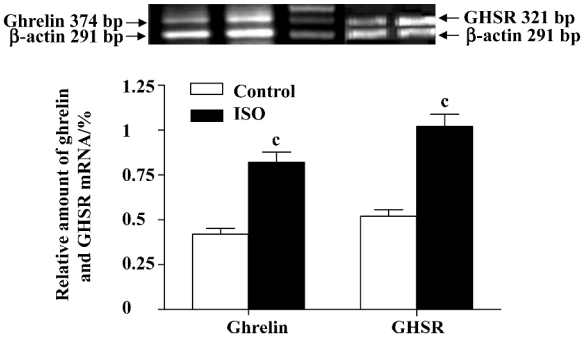

Figure 2. RT-PCR results for myocardial ghrelin and GHSR mRNA expression, and changes in ghrelin and GHSR mRNA levels in myocardia of rats. Ghrelin mRNA is seen as a 374 bp molecule, and GHSR mRNA as a 321 bp molecule. Rats were pretreated with ISO. n=3. Data are mean±SD. ^cP<0.01 *vs* control. Con, controls; ISO, ISO-treated rats.

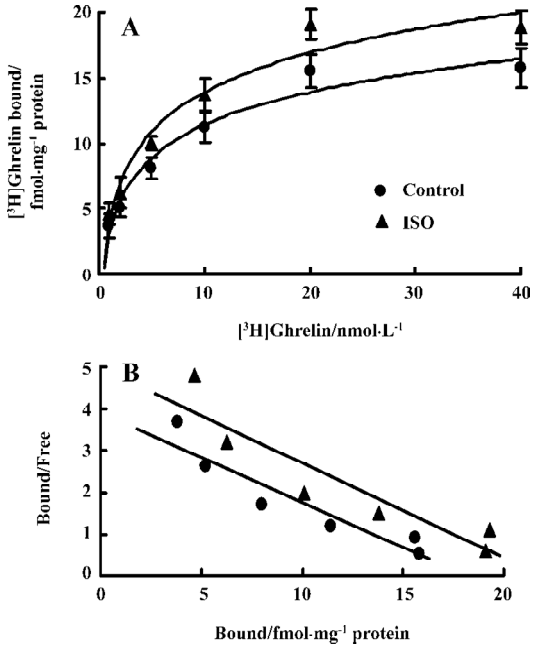

Figure 3. Representative saturation isotherms (A) and Scatchard plots (B) of [^{3}H]ghrelin binding to membranes from myocardia of rat hearts.

and CD content in myocardia was obviously reduced, by 36% and 30% (P<0.01), respectively, and in plasma by 27% and 20% (P<0.01), respectively. Myocardial fibrosis, shown by hydroxyproline level, was decreased by 40% (P<0.05; Table 2). Histological sections showed that the ISO-induced myocardial injury was obviously ameliorated by ghrelin therapy, with the area stained by collagen markedly decreased (Figure 1A, 1B).

Des-octanoyl ghrelin administration also ameliorated the ISO-induced myocardial injury. Compared with the ISO-alone group, ISO-treated rats showed lower heart weight and heart coefficient, decreased by 8% and 9%, respectively (P<0.01); higher +LV dp/dt_{max} and -LV dp/dt_{max} values, increased by 54% and 70% (P<0.01), respectively, and lower LVEDP, by 19% (P<0.01; Table 1). Myocyte damage, such as myocardial LDH leakage, was ameliorated (P<0.01), and MDA and CD content in myocardia was reduced, all by 12% (P<0.01). MDA and CD level in plasma was decreased by 19% and 17% (P<0.01), respectively. Fibrosis was relieved in myocardia, as shown by an 18% lower hydroxyproline level (P<0.05; Table 2). Histological sections showed ameliorated ISO-induced myocardial injury and collagen deposition (Figure 1A, 1B).

The cardioprotective effects of exogenous des-octanoyl ghrelin administration on ISO-induced myocardial injury, cardiac function suppression, cardiomegaly, collagen deposition and fibrosis were markedly weaker than those of equal doses of ghrelin (P<0.05 or P<0.01; Table 2, Figure 1).

Effect of ghrelin and des-octanoyl ghrelin on plasma GH level Compared with the control group, rats treated with ISO alone showed unaltered plasma GH levels (2.88±0.31 *vs* 2.82±0.22 ng/mL, P>0.05); ghrelin injection greatly increased the GH level, by 1.5-fold (P<0.01), but des-octanoyl ghrelin did not affect the plasma GH level (P>0.05; Figure 4).

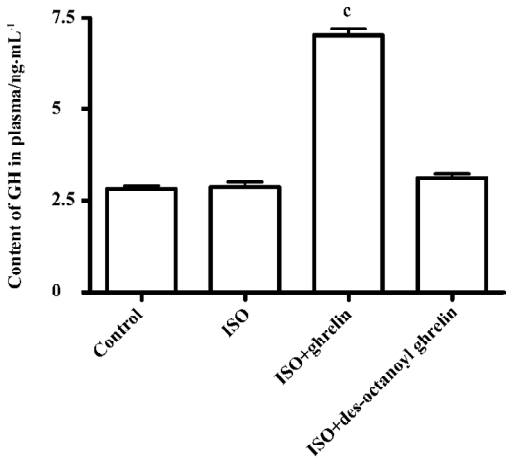

Figure 4. GH content in plasma in rats. n=3. Data are mean±SD. ^cP<0.01 *vs* control.

Discussion

Ghrelin is a strong gastrokinetic agent that links the endocrine control of energy balance and growth with the regulation of digestive function by activating its specific recep-

tor (GHSR-1a) in the central tissues. Binding sites specific for ghrelin and its mRNA expression exist in cardiovascular tissues, which indicates that the cardiovascular system expresses the components of the ghrelin/GHSR axis, which may have an important autocrine/paracrine function in maintaining circulatory homeostasis. Intravenous injection of ghrelin has been shown to elicit dose-related decreases in arterial pressure and heart rate in conscious rabbits[21]. Treatment with ghrelin causing a direct relaxation in rat thoracic aortic rings[22] and human internal mammary arteries[23] indicated that ghrelin is a vasorelaxing factor. Enomoto and colleagues[24] showed that subcutaneous administration of 3 doses of ghrelin (1, 5 or 10 μg/kg) caused a dose-dependent increase in the LV ejection fraction in healthy volunteers, which was still apparent 60 min after ghrelin injection; in patients with chronic heart failure, an intravenous infusion of human ghrelin (0.1 mg/kg per min) significantly increased cardiac and stroke volume index[25]. These results suggest that ghrelin could be an endogenous factor with potent protective effects against cardiovascular disease.

Over-release of catecholamines is an important factor related to myocardial impairment in many cardiovascular diseases, such as myocardial ischemia, hypertrophy and heart failure. We found that treatment with high doses of ISO for 10 d resulted in severe myocardial injury, including cardiomegaly, infarction-like necrosis and massive fibrosis, as did Rona *et al*[13]. Cardiac function was severely inhibited, lowering the values of ±LV dp/dt_{max} and elevating the LVEDP. The increase in LDH activity in plasma indicated the leakage of myocardial intracellular enzymes, and the increased MDA and CD content in myocardia and plasma indicated excessive products from lipid peroxidation. The increased hydroxyproline content in myocardia and picrosirius red-stained myocardial sections indicated myocardial fibrosis. In ISO-treated rats, the mRNA levels of ghrelin and GHSR in myocardia were elevated, the [^{3}H]ghrelin B_{max} of the sarcolemmal membrane was increased, and the ir-ghrelin content in plasma and myocardia was increased, with a negative correlation between ghrelin content and cardiac function. Furthermore, exogenous administration of ghrelin effectively ameliorated the ISO-induced myocardial injury, cardiac function inhibition, cardiomegaly, collagen deposition and fibrosis. These results indicate a role for ghrelin as an endogenous cardioprotective factor.

Various agents affecting endocrine and metabolism participate in the regulation of expression, synthesis and secretion of ghrelin. Food intake, obesity and positive energy balance downregulate ghrelin level, whereas starvation and nutritional disturbance upregulate ghrelin level; insulin, growth hormone release inhibiting hormone and gastrone restrain the expression of ghrelin. However, little is known about how ghrelin and GHSR are regulated when produced and secreted in local cardiovascular tissues. In both rats and humans, the ghrelin gene, located at 3p26–p25, is made up of 4 exons and 3 introns, and the precursors contain 117 amino acids (preproghrelin). Kishimoto and colleagures[26] cloned the human ghrelin gene and characterized the 5V-flanking region, from -2000 to -1 upstream of the translation start site. The gene contained a TATATAA element and putative binding sites for several transcription factors, but not a typical GC or CAAT box. Glucagon and its second messenger cAMP enhanced the promoter activity. From our work, the upregulated myocardial ghrelin and its receptor during ISO-induced myocardial injury needs further study.

Ghrelin is a multifunctional peptide, and is crucial in regulating energy homeostasis. It induces positive energy balance by stimulating food intake, while decreasing fat utilization. In cardiovascular tissue, ghrelin has direct vasodilator and inotropic effects[22,23]. Ghrelin increases endothelial nitric oxide synthase (eNOS) expression and nitric oxide production[24], and interferes with endothelin-1 synthesis and release[9]. Our present work showed that treatment with ghrelin or des-octanoyl ghrelin ameliorated ISO-induced myocardial injury, and the protective effects of ghrelin were at least in part mediated by its hemodynamic potency.

It is well known that oxidative stress injury and inflammatory reactions are the main mechanisms of high-dosage ISO-induced myocardial injury. Li and colleagues[27] confirmed that ghrelin inhibited proinflammatory cytokine production, mononuclear cell binding, and nuclear factor-κB activation in human endothelial cells *in vitro* and endotoxin-induced cytokine production *in vivo*. In ISO-induced myocardial injury and in ischemia/reperfusion-induced injury in isolated rat hearts, Chang and coworkers[9] found that ghrelin effectively scavenged oxygen free radicals and inhibited lipid peroxidation injury. In our work, ghrelin and des-octanoyl ghrelin diminished leukocytic infiltration and the generation of MDA and CD, which showed that the anti-inflammatory and anti-lipid peroxidation potency of ghrelin is involved in heart protection.

Ghrelin is the only endogenous ligand of GHSR, and ghrelin-activated GHSR-1a in the pituitary gland and hypothalamus induces GH release. Many reports have found that GH administration increases cardiac performance[28], deflates infarct size and reduces unfavorable remodeling. All these effects strongly slow the invasion of heart failure[29]. Imanishi and coworkers[30] reported that GH had an anti-

fibrotic effect on cardiac remodeling through a downregulation of tumor growth factor-β (TGF-β) signaling via dephosphorylation of p38 mitogen-activated protein kinase. To determine the cardioprotective mechanism of ghrelin, we compared the roles of ghrelin and des-octanoyl ghrelin in the present study. Des-octanoyl ghrelin is produced by removing octanoic acid on Ser3 from ghrelin, and lacks GH-releasing activity. Both ghrelin and des-octanoyl ghrelin effectively ameliorated ISO-induced myocardial injury, cardiac function inhibition, cardiomegaly, collagen deposition and fibrosis. The protective effect of des-octanoyl ghrelin was much weaker than that of ghrelin, which indicates that ghrelin plays its cardioprotective role in both a GH-dependent and -independent manner.

In summary, ISO-induced myocardial injury results in a compensatory increase in cardiac ghrelin and GHSR mRNA level and receptor binding capacity. Administering exogenous ghrelin and des-octanoyl ghrelin effectively attenuated ISO-induced myocardial injury, potentiated cardiac function, and ameliorated cardiac fibrosis, but the protection afforded by des-octanoyl ghrelin was markedly weaker than that of ghrelin, showing that ghrelin plays its cardioprotective role in a GH-dependent and -independent manner. Ghrelin could be an endogenous cardioprotective factor and a novel target in the prevention of and/or therapy for cardiovascular disease.

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