Ghrelin protects myocardium from isoproterenol-induced injury in rats[1]

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ABSTRACT

AIM: To investigate the cardiac protective effects of ghrelin in rat with myocardial injury induced by isoproterenol (ISO). **METHODS:** Rats were subcutaneously injected ISO 40 mg·kg^{-1}·d^{-1} with or without ghrelin 1 or 10 nmol·kg^{-1}·d^{-1} for 2 d. Hemodynamic parameters including mean arterial blood pressure and left ventricular pressure were measured at 12 h after the last injection with ISO and/or ghrelin. Plasma lactate dehydrogenase (LDH) activity, plasma and myocardial contents of malondialdehyde (MDA), and conjugated diene were measured. Plasma ghrelin and endothelin-1 levels were assayed using radioimmunoassay methods. Endothelin-1 and ghrelin mRNA were determined using RT-PCR. **RESULTS:** About 45 % (5/11) of rats after treatment with ISO alone died during experimental periods. However, no rats died after administration with ghrelin 10 nmol·kg^{-1}·d^{-1} (0/11, P<0.05). Ghrelin also obviously ameliorated the hemodynamic disturbance in rats induced by ISO. The plasma LDH activity, contents of myocardial and plasma MDA, and conjugated diene level in plasma in ISO+G10 nmol·kg^{-1}·d^{-1} group were decreased by 28 %, 34 %, 73 %, and 38 % compared with those of ISO group (all P<0.01) respectively. ISO-induced endothelin-1 mRNA over-expression was inhibited and endothelin-1 level in plasma were inhibited by ghrelin 1 and 10 nmol·kg^{-1}·d^{-1}. The ghrelin levels in plasma and ghrelin mRNA in myocardium were increased in the rats after injection of ISO. The plasma ghrelin level was further increased after ghrelin administration. **CONCLUSION:** Ghrelin has a protective effect against ISO-induced myocardial injury.

INTRODUCTION

Ghrelin, a novel 28-amino acid peptide that was principally released from the stomach, has been identified as an endogenous ligand for the growth hormone secretagogue receptor (GHSR). Mouse ghrelin differed from human ghrelin in two of its 28 residues, with lysine replacing arginine at position 11 and alanine replacing valine at position 122[1]. GHSR-1a, a G protein-coupled receptor mainly expressed in the pituitary and the hypothalamus, was thought to mediate the endocrine actions of ghrelin, including its potent growth hormone secreting effects. Two forms of ghrelin were present in human plasma: the unmodified peptide and a less abun-

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dant acylated version, in which octanoic acid was attached to the third residue, a serine, via an ester linkage[2]. Although ghrelin is essentially a gastric hormone, it was expressed ubiquitously and circulated in plasma at a concentration of approximately 100 pmol/L in healthy humans[3]. Besides having a stimulatory effect on growth hormone (GH) secretion, ghrelin administration was shown to induce positive energy balance by stimulating food intake while decreasing fat utilization through GH-independent mechanisms[4]. Moreover, cardiovascular tissues were rich in ghrelin receptors, and recent studies showed that ghrelin possessed a variety of cardiovascular effects independent of GH secretion, including increased myocardial contractility and vasodilatation, or protection from heart failure induced by myocardial infarction or septic shock *in vivo*[5-7]. Ghrelin levels were elevated in the setting of congestive heart failure and in cachetic patients with congestive heart failure, the mean plasma concentration of ghrelin was reported to be 237 pmol/L[5]. Based on these observations, we raised the hypothesis that ghrelin might have a protective effect on heart injured by ischemia. In this study, a myocardial injury model induced by isoproterenal (ISO) was used to investigate the cardiac effects of ghrelin and its mechanism.

MATERIALS AND METHODS

Reagents and animal This study protocol was approved by The Council on Animal Care at the Peking University. Male Sprague Dawley rats (Clean grade, Certificate No SCXK 11-00-0008) weighing 200-250 g were supplied by Animal Center, Health Science Center, Peking University. Synthetic rat ghrelin and ghrelin RIA assay kit were produced by Phoenix Pharmaceuticals (CA, USA). ISO and 1,1,3,3-tetraethyloxy-propane were purchased from Sigma Company (MO, USA). Other chemicals and reagents were of analytical grade. The specific primers for endothelin-1, ghrelin, and β-actin were synthesized by SBS Co (Beijing, China).

Experimental protocol with myocardial injury with ISO[14] Rats were randomly divided into 5 groups: control group (C group, n=7), ghrelin alone group (G group, n=7), ISO group (n=11), low dosage of ghrelin treated group (ISO+GL group, n=11) and high dosage of ghrelin treated group (ISO+GH group, n=11). Rats in ISO group were injected with ISO (40 mg·kg^{-1}·d^{-1}, bid, sc). Rats in G group were injected with ghrelin of 10 nmol·kg^{-1}·d^{-1} (bid, sc). Rats in ISO+GL and ISO+GH groups were subcutaneously injected with ghrelin of 1 and 10 nmol·kg^{-1}·d^{-1} (bid, sc), respectively, in addition to administration with ISO as in ISO group. Control rats were given 0.9 % NaCl (bid, sc). All rats were subjected to treatment of 2 d (4 injections). Twelve hours after the last injection, hemodynamic parameters were measured in all surviving rats anesthesized with sodium pentobarbital (40 mg/kg, ip). A catheter filled with heparin saline (500 kU/L) was inserted into the right common carotid artery for measurement of mean arterial pressure (MAP), and was further implanted into the left ventricle to record the LV dp/dt_{max}, left ventricular end diastolic pressure (LVEDP), and heart rate (HR). Arterial blood was then collected and plasma was separated. The hearts were removed for histological analysis and RT-PCR for ET-1 and ghrelin.

Assay of LDH activity, contents of MDA, and conjugated diene LDH activity was measured by automatic biochemistry analyzer. The contents of lipid-peroxidation product, MDA, in myocardium and plasma were measured with the thiobarbituric acid reaction. Standard MDA was prepared by acid hydrolysis of 1,1,3,3-tetraethyloxypropane[8]. Conjugated diene content in plasma was determined according to method reported by Waller *et al*[9].

Radioimmunoassay of ET-1 and ghrelin level in plasma The plasma acidulated with 0.1 mol/L acetic acid was applied to a Sep-Pak C18 cartridge (Millipore-Waters, Milford, MA, USA). After washing the cartridge with 10 % CH_3CN in 0.1 % trifluoroacetic acid, the absorbed materials were eluted with 50 % CH_3CN in 0.1% trifluoroacetic acid, lyophilized, and stored at -70 ºC until assayed. ET-1 and ghrelin in plasma were measured using a specific RIA kit for ET-1[10] and ghrelin[7] respectively. Rabbit polyclonal anti-ET-1 and anti-ghrelin antibodies were used for RIA in these kits. The IC_{50} for rat endothelin was 14.6 pg/tube. The cross-reactivity with rat endothelin was 100 %, and that with ET-2, ET-3, and angiotensin II was 3.5 %, 28 %, and 0, respectively. The sensitivity of IC_{50} for rat ghrelin was 6.95 pmol/tube, and binding was 35 % in this assay. All assays included 12 plasma control samples from common stock solutions, which were frozen in aliquots at the beginning of the study, in order to normalize each test for inter-assay variability. Based on these controls, the intraassay coefficient of variation was 8.7 % and the interassay coefficient of variation was 14.6 % (n=10). No cross-reactivity was seen with leptin, orexin A and B, neuropeptide Y, galanin,

or vasoactive intestinal polypeptide, assessed with doubling dilutions from 1 to 100 μg/L.

RT-PCR for ET-1[11] and ghrelin mRNA[12] Total RNA from heart was extracted with Trizol (Gibco BRL, Rockville, MD) reagent. RT-PCR was performed in a total volume of 25 μL. The PCR product was separated in a 1.5 % agarose gel, and stained with ethidium bromide. The ratio of optical density ET-1 and ghrelin mRNA to β-actin was measured using the Gel Documentation System (Bio-Rad, Hercules, CA). The primers for ET-1 as: sense: 5'-GGTCTTGATGCTGTTGCTGA-3', anti-sense: 5'-GAGCTGAGAAGGAAGTGCAGA-3'. The RT-PCR condition is: denaturation at 95 ºC for 5 min followed by 30 cycles at 94 ºC for 30 s, 60 ºC for 30 s, and 72 ºC for 40 s. The primers for ghrelin is: sense: 5'-TTGAGCCCAGAGCACCAGAAA-3', anti-sense: 5'-AGTTGCAGAGGAGGC AGAAGC T-3', and the RT-PCR condition was: denaturation at 95 ºC for 5 min followed by 30 cycles at 94 ºC for 5 min, 94 ºC for 30 s, 55 ºC for 40 s, 72 ºC for 40 s, and finally extension at 72 ºC for 5 min. The primers for β-actin was: sense: 5' ATCTGGCACCACACCTTC-3', anti-sense: 5'-AGCCAGGTCCAGACGCA-3', and the RT-PCR condition is: denaturing at 94 ºC for 30 s, annealing at 55 ºC for 30 s, and extension at 72 ºC for 30 s for 25 cycles.

Statistical analyses Data were expressed as mean±SEM. One way analysis of variance (ANOVA) was performed when more than two groups were compared, and when significant (P<0.05), Newman-Keuls test was applied to test for the differences between individual groups. P<0.05 was considered statistically significant. X^2 test was used for comparisons of mortality rate among groups. P<0.05 was considered statistically significant.

RESULTS

Ghrelin reduced the mortality rate in the ISO-treated rats All rats in the C and the G group remained alive during the experimental period. About 45 % (5/11) rats in the ISO group died, whereas the mortality rate was only 18 % (2/11) in ISO+G1 nmol·kg^{-1}·d^{-1} group (P>0.05, compared with ISO group) and 0 % (0/11) in ISO+G10 nmol·kg^{-1}·d^{-1} group (P<0.05).

Ghrelin potentiated cardiac function in the rats treated with ISO The rat cardiac function was significantly inhibited by ISO injection, which was indicated by increased LVEDP (+86 %, P<0.01). The +LV$dp/dt_{max}$ and -LV$dp/dt_{max}$ were reduced by 48 % (P<0.05) and 38 % (P<0.05), compared with those of control group respectively. Treatment with ghrelin (10 nmol·kg^{-1}·d^{-1}, 2 d) alone did not affect the cardiac function in normal rats. The cardiac ventricular pressure (LVEDP, +LV$dp/dt_{max}$ and -LV$dp/dt_{max}$) was not statistically different when compared with control group (all P>0.05). However, treatment with ghrelin (1 and 10 nmol·kg^{-1}·d^{-1}, 2 d) in ISO+GL and ISO+GH groups effectively inversed the inhibitory effects on cardiac function induced by ISO. The values of LVEDP and +/-LV$dp/dt_{max}$ were significantly increased compared with those in ISO alone group (all P<0.01). There was no statistical difference between ISO+GL and ISO+GH groups in improvement of cardiac function (all P>0.05). In this study, the MABP was not statistically different between various groups. Administration with ISO increased HR (P<0.05 *vs* control group), and treatment with ghrelin did not ameliorate tachycardia induced by ISO (P>0.05, Tab 1).

Ghrelin attenuated myocardium injury induced by ISO LDH is a sensitivity enzyme indicating the cell

Tab 1. Effects of ghrelin on mean artery blood pressure (MABP) and heart function induced by isoproterenol. Mean±SEM. $^b P$<0.05, $^c P$<0.01 *vs* control group. $^f P$<0.01 *vs* ISO group.

Groups	MABP/mmHg	HR/mmHg	LVEDP/mmHg	+LV$dp/dt_{max}$	–LV$dp/dt_{max}$
Control (n=7)	82±4	387±17	6±2	3381±172	2808±192
G 10 nmol·kg^{-1}·d^{-1}(n=7)	84±4	394±11	4±2	3197±282	2140±135
ISO(n=6)	82±5	422±6^b	24±6^c	1761±183^b	1750±179^b
ISO+G1 nmol·kg^{-1}·d^{-1}(n=9)	80±7	422±17	2±1^f	3664±266^f	2741±327^f
ISO+G10 nmol·kg^{-1}·d^{-1}(n=10)	82±5	401±9	4±1^f	4038±166^f	2657±231^f

±LV$dp/dt_{max}$: left ventricular dp/dt maximum (mmHg·s^{-1}). HR: heart rate; LVEDP: left ventricular end diastolic pressure.

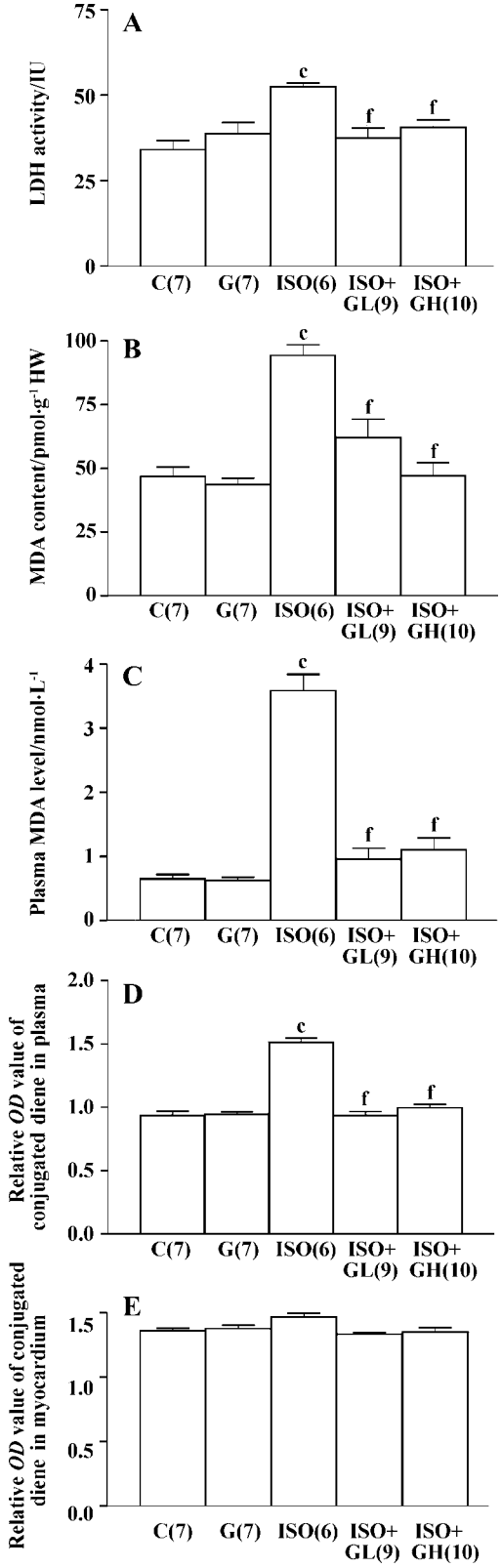

Fig 1. Effects of ghrelin on plasma LDH activity (A), MDA content in myocardium (B), plasma MDA content (C), conjugated diene in plasma (D), and conjugated diene in myocardium (E). Mean±SEM. $^{c}P<0.01$ *vs* Con group. $^{f}P<0.01$ *vs* ISO group. The number in parentheses represents the rat number in each group.

injury. In normal conditions, it exists in intracellular compartment, and will leak into plasma when cells are injured. In this study, the plasma LDH activity was significantly increased by 54 % in rats of ISO group compared with that of control group (Fig 1A, $P<0.01$). Injection of ISO induced an increase in MDA contents in myocardium (Fig 1B) and plasma (Fig 1C), that were 2-fold and 5.5-fold more than those of control group, respectively (all $P<0.01$). Plasma conjugated diene, another lipid peroxide product, was also increased by 1.6-fold in ISO group compared with control group (Fig 1D, $P<0.01$), but the conjugated diene in myocardium was not altered in ISO groups (Fig 1E, $P>0.05$). Ghrelin alone did not affect the plasma LDH activity, contents of MDA, and conjugated diene in comparison to those of control group ($P>0.05$). Interestingly, administration with ghrelin significantly attenuated myocardial injury induced by ISO. The plasma LDH activity, contents of myocardial and plasma MDA, and conjugated diene level in plasma in ISO+G10 nmol·kg^{-1}·d^{-1} group were decreased by 28 %, 34 %, 73 %, and 38 % compared with those of ISO group (all $P<0.01$) respectively. There was no statistical difference between ISO+G1 nmol·kg^{-1}·d^{-1} and ISO+G10 nmol·kg^{-1}·d^{-1} groups in term of myocardial injury (all $P>0.05$).

Ghrelin affected myocardial ET-1 mRNA expression and plasma ET-1 level The plasma ET-1 level was significantly increased by 58 % in ISO group compared with control group ($P<0.01$). Exogenous administration with ghrelin 1 and 10 nmol·kg^{-1}·d^{-1} significantly reduced plasma ET-1 level by 24 % and 30 % compared with ISO group ($P<0.01$, Fig 2A). The RT-PCR results showed that ISO significantly increased ET-1 mRNA expression in myocardium by 2.8-fold compared with control group ($P<0.01$). Treatment with ghrelin obviously inhibited ISO-stimulated expression of myocardial ET-1 mRNA (Fig 3A).

Effects of ISO on myocardial ghrelin mRNA level and plasma ghrelin content Exogenous administration with ghrelin significantly increased plasma ghrelin level (Fig 2B, $P<01$ *vs* ISO group). There was a few ghrelin mRNA expression in normal myocardium. Treatment with ISO significantly induced the expression of myocardial ghrelin mRNA and increased the plasma ghrelin level when compared with control groups ($P<0.05$, Fig 3B).

DISCUSSION

Ghrelin, a 28-amino-acid peptide predominantly

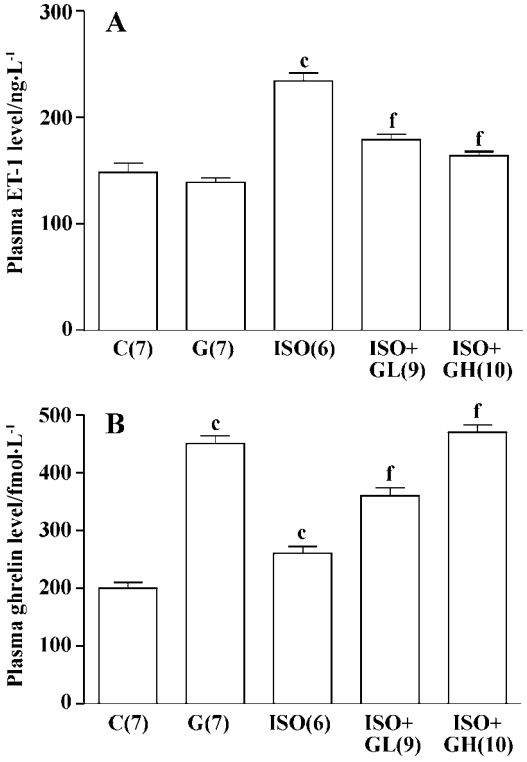

Fig 2. Plasma ET-1(A) and ghrelin(B) level. Mean±SEM. $^{c}P<0.01$ *vs* Con group. $^{f}P<0.01$ *vs* ISO group. The number in parentheses represents the rat number in each group.

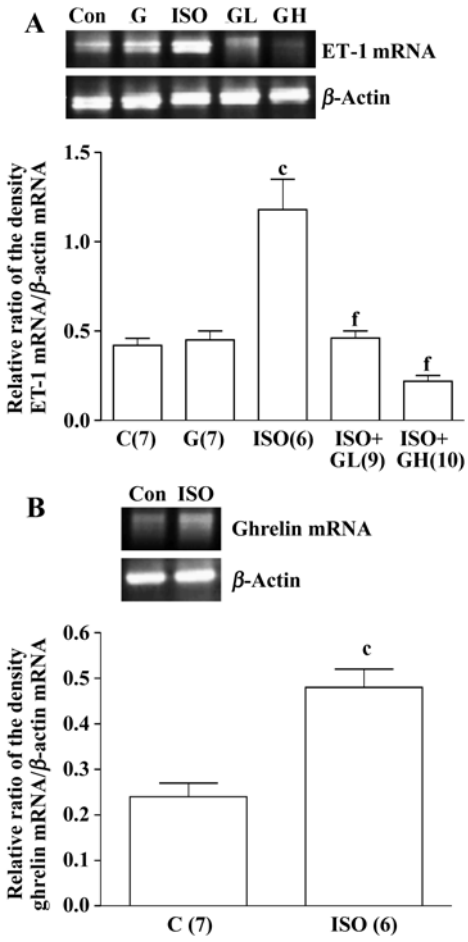

Fig 3. ET-1 mRNA (A) and ghrelin mRNA (B) expression in myocardium in different groups. The column figure is the ratio of ET-1 mRNA/β-actin mRNA (A) and ghrelin mRNA/ β-actin mRNA (B). Mean±SEM. $^{c}P<0.01$ *vs* control group. $^{f}P<0.01$ *vs* ISO group. The number in parentheses represents the rat number in each group.

produced by the stomach, strongly stimulated growth hormone release when they activated ghrelin receptor, known as a G-protein coupled receptor of the growth hormone secretagogue receptor. The ghrelin receptor was widely distributed in the body. In addition to pituitary gland, cardiovascular tissues such as myocardium, aorta, coronary artery and vein were rich in ghrelin receptors[13], suggesting that ghrelin could directly exert cardiovascular effects by growth hormone-independent mechanisms. Here, we demonstrated that the plasma ghrelin level was increased in rats after ISO-induced myocardial injury, and that administration of ghrelin decreased mortality, improved hemodynamic disturbance, and attenuated myocardial injury in rats treated with ISO. These findings suggested that ghrelin could be an endogenous protective factor against myocardium damage.

Overdose of ISO was a commonly used agent to induce myocardial injury through catecholamine intoxication and oxidative stress[14]. In this study, the subcutaneous injection of ISO resulted in a significant myocardial injury and cardiac dysfunction. The LVEDP was significantly increased. The +LV$dp/dt_{max}$, an index of myocardial contractility, and -LV$dp/dt_{max}$, which reflected the ability of the heart to relax, were all decreased in ISO group. ISO group showed LDH leakage and formation of MDA and conjugated diene. Administration with ghrelin resulted in obviously improved cardiac function and myocardial damage induced by ISO. It was found that both +LV$dp/dt_{max}$ and -LV$dp/dt_{max}$ was increased, and LVEDP was decreased by ghrelin treatment, LDH leakage was reduced, and generation of lipid peroxide product of MDA and conjugated diene was lowered.

The mechanisms by which ghrelin improved myocardial function and metabolism following ISO-damage were unclear. The peptide ET-1 was a potent and long-lasting vasoconstrictor and mytogen[15], and evidenced as an important pathogenetic factor of cardio-

vascular diseases, including ISO-induced myocardial injury[21]. It was reported that ghrelin (0.1-300 nmol/L) potently dilated ET-1 10 nmol/L-induced human arteries constrictions, indicating that ghrelin was an effective and natural antagonist of the ET-1[16]. The results in this study showed that the plasma ET-1 level and myocardial ET-1 mRNA expression were significantly increased in the ISO-treated rats, and it was in accordance with previous report[21]. Interestingly, exogenous supplement with ghrelin significantly reduced plasma ET-1 level and myocardial ET-1 mRNA expression, indicating that the protective effects of ghrelin was at least in part mediated through interfering ET-1 synthesis and release.

In the present study, treatment with ISO significantly induced the expression of myocardial ghrelin mRNA and increased the plasma ghrelin level. Exogenous administration with ghrelin significantly increased plasma ghrelin level. The endocrine activity of ghrelin was thought to be entirely dependent on its acylation and subsequent interaction with GHSR-1a[13]. In addition to GHSR-1a, CD36, a multifunctional glycoprotein expressed in cardiomyocytes, potentially served as a myocardial ghrelin receptor. CD36 was recently identified as a binding site for hexarelin, synthetic growth hormone secretagogue peptide, in myocardial tissue, and the effects of hexarelin on coronary perfusion pressure were abolished in hearts from CD36-null mice [19]. Whether CD36, GHSR-1a, or some other receptor mediates the cardioprotective effects of ghrelin following ISO intoxication remains to be determined. Rather, ghrelin more likely exerted its cardioprotective effects through activation of cellular signal transduction cascades. For instance, ghrelin inhibited H9c2 cardiomyocyte apoptosis *in vitro* by modulating extracellular signal-regulated kinase-1/2 and Akt serine kinases[20]. In addition, ghrelin recognized high affinity binding sites on H9c2 cardiomyocytes, which did not express GHSR-1a. Finally, both MK-0677 (a nonpeptidyl synthetic growth hormone secretagogue) and hexarelin recognized a common binding site, and both compounds inhibited cell death and activated MAPK and Akt in H9c2 cells[20].

The results in this study showed that as low as ghrelin 1 nmol·kg^{-1}·d^{-1} significantly attenuated the myocardial injury. It was unexpected that ghrelin 10 nmol·kg^{-1}·d^{-1} was not more effective than 1 nmol·kg^{-1}·d^{-1} administration, suggesting that ghrelin had potent cardiovascular protective effects. Considering that there are amount of ghrelin receptor in cardiovascular system, ghrelin may has important cardioprotective significance in physiological and pathophysiological conditions. Exogenous administration with ghrelin may serve as a novel therapeutic strategy for ischemic cardiovascular diseases.

REFERENCES

1 Banks WA, Tschop M, Robinson SM, Heiman ML. Extent and direction of ghrelin transport across the blood-brain barrier is determined by its unique primary structure. J Pharmacol Exp Ther 2002; 302: 822-7.

2 Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 1999; 402: 656-60.

3 Shiiya T, Nakazato M, Mizuta M, Date Y, Mondal MS, Tanaka M, *et al*. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. J Clin Endocrinol Metab 2002; 87: 240-4.

4 Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature 2000; 407: 908-13.

5 Nagaya N, Uematsu M, Kojima M, Ikeda Y, Yoshihara F, Shmizu W, *et al*. Chronic administration of ghrelin improves left ventricular dysfunction and attenuates development of cardiac cachexia in rats with heart failure. Circulation 2001; 104: 1430-5.

6 Chang L, Du JB, Gao LR, Pang YZ, Tang CS. Effect of ghrelin on septic shock in rats. Acta Pharmacol Sin 2003; 24: 45-9.

7 Chang L, Zhao J, Yang J, Zhang ZK, Du JB, Tang CS. Therapeutic effects of ghrelin on endotoxic shock in rats. Eur J Pharmacol 2003; 473: 171-6.

8 Askawa T, Matashushita S. Thiobarbituric acid test for detecting lipid peroxide. Lipids 1979; 14: 401-6.

9 Waller RL, Recknagel RO. Determination of lipid conjugated dienes with tetracyanoethylene-14C: significance for study of the pathology of lipid peroxidation. Lipids 1977; 12: 914-21.

10 Li WG, Li ZB, Gao YQ. Comparison of the changes of cardiac endogenous active peptides in exercise-induced and hypertensive hypertrophic hearts in rats. Chin J Sports Med 2000; 19: 27-8.

11 Savage P, Jeng AY. Quantitation of plasma endothelins. Anal Biochem 1993; 213: 434-6.

12 Ariyasu H, Takaya K, Hosoda H, Iwakura H, Ebihara K, Mori K, *et al*. Delayed short-term secretory regulation of ghrelin in obese animals: evidenced by a specific RIA for the active form of ghrelin. Endocrinology 2002; 143: 3341-50.

13 Gnanapavan S, Kola B, Bustin SA, Morris DG, McGee P, Fairclough P, *et al.* The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. J Clin Endocrinol Metab 2002; 87: 2988.

14 Shi YR, Bu DF, Qi YF, Gao L, Jiang HF, Pang YZ, *et al*. Dysfunction of myocardial taurine transport and effect of taurine supplement in rats with isoproterenol-induced myo-

cardial injury. Acta Pharmacol Sin 2002; 23: 910-8.
15 Xu JT, Tang CS, Hu DY, Pang YZ. Effect of endothelin-endothelin receptor on thrombin-induced vascular smooth muscle cell proliferation. Chin J Microcirc 2002;12: 38-9.
16 Wiley KE, Davenport AP. Comparison of vasodilators in human internal mammary artery: ghrelin is a potent physiological antagonist of endothelin-1. Br J Pharmacol 2002; 136:1146-52.
17 Li X, Wang XB, Li L, Pang YZ, Su JY, Tang CS. Role of endothelin in N^G-nitric-*L*-arginine methylester-induced hypertension in rats. Chin J Pathophysiol 1999; 15: 230-2.
18 Li ZB, Gao YQ, Li WG, Yang P, Tang CS. Expression of endothelin-1 and its gene in myocardium of exercise-induced and hypertensive hypertrophied heart. Chin J Sports Med 2000; 19: 25-6.
19 Locatelli V, Rjossoni G, Schweiger F, Torsello A, Gennaro DE, Colonna V, *et al*. Growth hormone-independent cardioprotective effects of hexarelin in the rat. Endocrinology 1999; 140: 4024-31.
20 Bodart V, Febbraio M, Demers A, McNicoll N, Pohankova P, Perreault A, *et al*. CD36 mediates the cardiovascular action of growth hormone-releasing peptides in the heart. Circ Res 2002; 90: 844-9.
21 Li BY, Jin Y, Wang JH. Relationship between the endothelin-nitric oxide system and collagen metabolism in ischemia myocardium. Chin J Clin Exp Pathol 1999; 15: 57-9.