

**Effect of Ghrelin on Isoprenaline Induced Myocardial Infarction in
Rats**

By

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Abstract

AIM: To investigate the cardiac effects of ghrelin in rats with myocardial injury induced by isoprenaline (ISO).

METHODS: Forty healthy adult male albino rats weighting 180 – 200 gm were included in this study. The rats were randomly classified into 4 equal experimental groups; **Group**

I (Control group): Received no medication and given free access to food and water.

Group II (ghrelin group): Received Ghrelin (Gh) only in a dose of 10nmol/kg subcutaneous (S.C) for 2 days. **GroupIII (isoprenaline group):** Received single dose of

isoprenaline (ISO) 75mg/kg intraperitoneal (i.p.) for induction of myocardial infarction (M.I). **Group IV(ghrelin treated group):** Received ghrelin in adose of 10 nmol/kg/day

SC for 2 days before induction of myocardial infarction with ISO (as in ISO group).Thirty minutes after induction of M.I., a blood sample was collected from the right ventricle for estimation of plasma CK-MB, ghrelin and endothelin-1 levels and heart was excised for measurement of infarction size as percentage of left ventricle.

RESULTS: About 20 % of rats after treatment with ISO alone died during experimental periods. However, no rats died after administration with ghrelin 10 nmol/Kg/d.

Endothelin-1 and CK-MB level in plasma was decreased by ghrelin (10 nmol/kg) with significant reduction of the infarction size. Plasma ghrelin level was increased in the rats after 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 peptide of 28 amino acids, was first reported by Kojima in rats' and human stomachs in 1999 [1]. Two thirds of the circulating ghrelin originates in the X/A-like cells in the oxyntic mucosa of the stomach and the rest is produced by cells of the small intestine [2]. In addition, a smaller amount of ghrelin is also produced by other organs such as heart, lung, kidney, pancreas, placenta, lymphatic tissue, gonads, thyroid, adrenal, pituitary, hypothalamus and some neoplastic tissues and cancer-cell lines [3, 4, 5, 6]. Ghrelin is also detected in cerebrospinal fluid but at much lower concentration (about 1/30 – 1/40 of that of circulating ghrelin) [7]. Ghrelin was initially identified to be a strong stimulant for the release of GH that has been identified as an endogenous ligand for the GH secretagogues receptor (GHS-R), GHSR-1a, a G protein-coupled receptor mainly expressed in the pituitary and the hypothalamus [6, 8]. In subsequent research, ghrelin and its various receptors were found everywhere in many organs and tissues. Moreover, they participate in the regulation of appetite, energy, bodyweight, metabolism of glucose and fat, as well as modulation of gastrointestinal, cardiovascular, pulmonary, immune functions, cell proliferation/apoptosis, antiinflammatory, reproductive and bone formation [9, 10]. Cardiovascular tissue is rich in ghrelin receptors[11]. The best investigated ghrelin receptor, GHS-R1a, GHS-R1b are highly expressed in human myocardium and aorta [6]. An unknown subtype of ghrelin receptor, distinct from GHS-R1a and GHS-R1b, is found in cardiomyocytes and endothelial cells. In addition, CD36 is expressed in rat and human cardiovascular tissues [12]. Recent studies showed that ghrelin possessed a variety of cardiovascular effects independent of GH secretion, including increased myocardial contractility and vasodilatation, protection from heart failure induced by myocardial infarction or septic shock, decrease cardiac injury induced by ischemia/reperfusion (I/R) or isoprenaline and reduce infarction size [13, 14]. Based

on these observations, we raised the hypothesis that ghrelin might have a protective effect on heart injured by ischemia.

Materials and Methods:

I-Chemicals used:

1. Synthetic rat ghrelin and ghrelin RIA assay kit were produced by Phoenix Pharmaceuticals (CA, USA).
2. ISO was provided as white powder from (Sigma Company, USA) and dissolved in distilled water.
3. Urethane: provided as white powder (Sigma, U.S.A.). It was dissolved in saline before use.
4. Creatine Kinase - MB kits: (Spinreact, Spain).
5. Triphenyltetrazolium chloride: provided as white powder (MP biomedical, France).

II- Animals used:

Experimental protocol for the study was approved by the ethics committee on animal experiments in Benha University. Forty healthy adult male albino rats weighting 180 – 200 gm. averaging 16 weeks old were brought from Experimental Animal Breeding Farm, Helwan –Cairo to be utilized in this study. Rats were grown on normal diet and water. At the time of experiment, the rats were randomly classified into 4 equal experimental groups. **Group I (Control group):** Received no medication and given free access to food and water. **Group II (Ghrelin group):** Received Ghrelin only in adose of 10nmol/kg SC for 2 days [15]. **Group III (ISO group):** Received single dose of isoprenaline 75mg/kgm injected intraperitoneal for induction of M.I. [16]. **Group IV (Ghrelin treated group):** Received ghrelin in adose of 10 nmol /kg SC for 2 days before induction of myocardial infarction with ISO (as in ISO group). The rats were anesthetized by urethane in a dose 1.25g/kg body weight dissolved in saline, half the dose was injected i.p. for a rapid action and the other half was injected S.C for a slow sustained action [17]

•**ET-1 and ghrelin level in plasma:** Were assayed using radioimmunoassay methods [18].

- **CK-MB estimation:** After separation of sera, CK- MB was determined using CK- MB reagent provided by Spinreact Company, Spain [19].
- **Measurement of infarction size:** At the end of the experiment, intracardiac blood sample withdrawn then the heart was excised and the excised beating heart was submerged in cold (8°C) 30 mmol KCl to achieve diastolic arrest. The right ventricle and both atria were excised to isolate the left ventricle (the septum and free wall). The left ventricle was then sectioned by a sharp surgical scissor into transverse slices, each of about 1.5mm thick. The slices were then stained in a 1.5% triphenyltetrazolium chloride (TTC) in phosphate buffer, PH 7.4, for 10-15 minutes at 37°C. The TTC stain form red color precipitates in the presence of intact dehydrogenase enzyme system. Areas of necrosis lost dehydrogenase activity and therefore fail to stain [20, 21]. The slices were washed with saline and then clear glass plates were placed over both sides of each slice. Epicardial and endocardial outlines as well as the TTC stained and non-stained areas were traced on clear plastic sheets. The plastic sheet was then fixed on an E.C.G paper and the small squares occupying the stained and non-stained areas were counted giving each in mm². The sum of the stained and the non-stained areas give the surface area of the whole heart slices. The surface area of the whole left ventricle was calculated by adding the surface areas of all cardiac section measured on E.C.G paper. The surface area of infract tissue in the whole heart was obtained by adding the surface area of infract tissue in all cardiac slices and the infraction size was calculated as percentage of the sum of infract areas to the sum of surface areas of all the slices [22].

RESULTS

Ghrelin reduced the mortality rate in the ISO treated rats. All rats in the control and the Gh group remained alive. During the experimental period, about 20 % rats in the ISO group died, whereas the mortality rate was 0 % in Gh+ISOgroup ($P < 0.05$) as compared with ISO group.

(Tab- 1): Shows the values as mean \pm standard deviation. Then P values were calculated using Student t- test.

	Control group	Gh group	ISO group	Ghtreatedgroup (Gh + ISO)
CK-MB (U/L)	234.38 \pm 33.53	246.25 \pm 29.12	1497.5 \pm 111.45	597.5 \pm 138.95
ET-1 (ng/L)	143.75 \pm 27.87	131.88 \pm 27.64	239.38 \pm 19.54	146.0 \pm 15.03
Gh (mmol/L)	197.75 \pm 17.98	458.75 \pm 23.72	266.25 \pm 24.16	485.0 \pm 23.75
Infarction size (% LV)	0.0	0.0	34.88 \pm 473	17.38 \pm 3.11

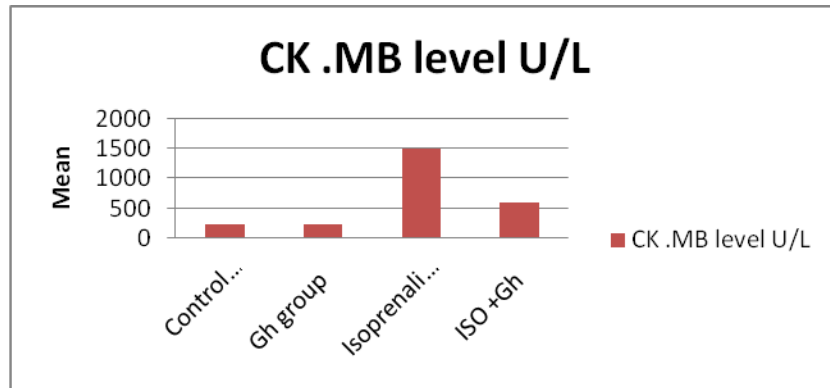
****Effect of Ghrelin on plasma CK-MB level (Table 1 & Fig. a):** In this study; the plasma CK-MB level was significantly increased in rats of ISO group compared with that of control group (Control group 234.38 \pm 33.43, in ISO group 1497.5 \pm 111.45; $P < 0.001$). Ghrelin alone did not affect the plasma CK-MB level, in comparison to those of control group ($P > 0.05$). Interestingly, administration of ghrelin before ISO significantly attenuated myocardial injury, that CK-MB level in plasma decreased from 1497.5 \pm 111.45 in ISO group to 597.5 \pm 138 in ISO + Gh group ($P < 0.001$).

****Effect of Ghrelin on plasma ET-1 level (Table 1 & Fig. b):** The plasma ET-1 level was significantly increased in ISO group compared to control group (Control group 143.75 \pm 27.87, ISO group 239 \pm 19.54; $P < 0.001$). Exogenous administration of ghrelin before ISO induced myocardial injury significantly reduced plasma ET-1 level (ISO group 239 \pm 19.54, Gh+ISO group 146.0 \pm 15.03; $P < 0.001$), while ghrelin alone did not affect the plasma ET-1 level in comparison to those of control group ($P > 0.05$).

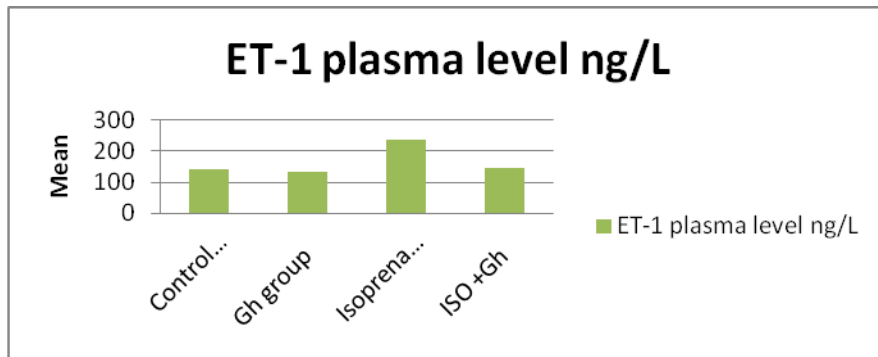
****Effects of ISO on plasma ghrelin level (Table 1 & Fig. c):** Exogenous administration with ghrelin alone significantly increased plasma ghrelin level from 197 \pm 17.48 in control group to 458.75 \pm 23.72 in Gh group ($P < 0.001$). Treatment with ISO significantly increased the plasma ghrelin level when compared with control group ($P < 0.001$). Administration of ghrelin before ISO significantly increased the plasma ghrelin

level when compared with ISO group (ISO group 266.25 ± 24.16 , Gh + ISO 485 ± 23.75 $P < 0.001$).

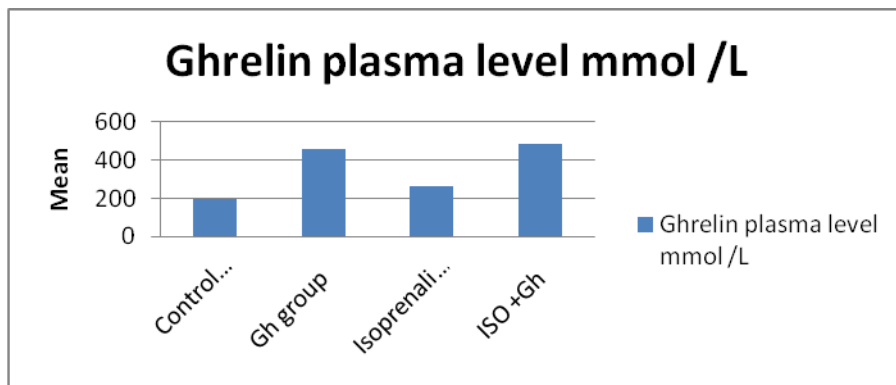
****Effects of Ghrelin on infarction size (Table 1& Fig. d):** Administration of ghrelin before ISO induced myocardial injury significantly decreased infarction size from 34.88 ± 4.75 in ISO group to 17.38 ± 3.11 in Gh + ISO group ($P < 0.001$).



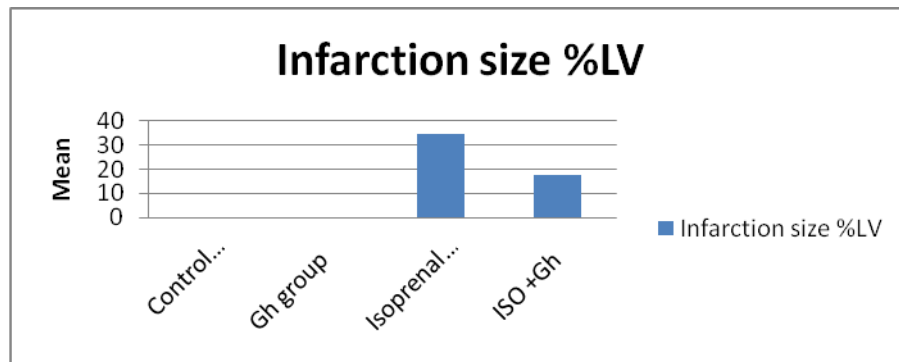
(Fig. a)



(Fig. b)



(Fig. c)



(Fig. d)

DISCUSSION

Ghrelin, is synthesized as a prehormone and then proteolytically processed to yield a 28-amino-acid peptide. This peptide predominantly produced by the stomach, strongly stimulates growth hormone release when they activates ghrelin receptor known as a G-protein coupled receptor mainly expressed in the pituitary and hypothalamus. The ghrelin receptor was widely distributed in the body. In addition to pituitary and hypothalamus, cardiovascular tissues such as myocardium, aorta, coronary artery and vein were rich in ghrelin receptors [7, 23] suggesting that ghrelin could directly exert cardiovascular effects by growth hormone independent mechanisms and likely involve binding to cardiovascular receptors, a process that is up regulated during myocardial injury [24].

In the present study, intraperitoneal injection of ISO resulted in a significant myocardial Injury. ISO group showed increase CK-MB level and infarction size. Overdose of ISO was a commonly used agent to induce myocardial injury through increase intracellular Ca^{++} , cAMP, increase heart rate, myocardial contractility and oxidative stress [5, 25]. Plasma ghrelin level was increased in rats after ISO-induced myocardial injury, perhaps due to a compensatory, self-protective mechanism. Moreover administration of exogenous ghrelin can protect against ISO induced myocardial injury [15] that administration of ghrelin resulted in obviously improved myocardial damage

induced by ISO by reducing CK-MB level and infarction size and decreased mortality [15, 26]. These findings suggest that ghrelin could be an endogenous protective factor against myocardium damage.

There are several mechanisms by which ghrelin improved myocardial function and metabolism following to myocardial damage. Ghrelin has been found to improve cardiac contractility to reduce the infarct size and to attenuate the reduction in left ventricular function induced by ischemia-reperfusion [23] Also Ghrelin exerted its cardioprotective effects by prevent cardiaomyocyte apoptosis, through GHSR-1a [12] that activates of extracellular signal regulated kinase (ERK) and Akt serine kinases in cardiomyocytes and endothelial cells [23, 27, 28, 29], in addition to its anti-inflammatory effect, that decrease interleukin- 6 (IL6) and tumour necrosis factor- α (TNF- α) through activation of GHSR-1a and AKT dependant pathway [29, 30] and antioxidative effect, through attenuation in vascular superoxide production and NADPH oxidase activity [31] and decrease the content of lipid peroxidation product malondialdehyde in myocardium of rat hearts [24, 32].

The results in this study showed that the plasma ET-1 level was significantly increased in the ISO-treated rats, peptide ET-1 was a potent and long-lasting vasoconstrictor and mytogen [33] and evidenced as an important pathogenic factor of cardiovascular diseases, including ISO-induced myocardial injury [34]. interestingly, exogenous supplement with ghrelin significantly reduced plasma ET-1 level , indicating that the protective effects of ghrelin was at least in part mediated through interfering ET-1 synthesis and release [15, 23]. It was found that ghrelin might act on the cardiac vagal afferent nerve, which sends projection to the nucleus of the solitary tract, resulting in a suppression of cardiac sympathetic activity and heart rate in rats with MI leading to improved left ventricular (LV) dysfunction and early cardiac remodeling, which is a major cause of subsequent heart failure and death following myocardial infarction (MI) [35, 36, 37]. Also it was found that ghrelin can markedly increase blood flow in mice with ischemia and reperfusion injury, that ghrelin improved endothelial function by increasing the nitric oxide production using a signaling pathway involving GHS-R 1a, phosphatidylinositol-3-kinase (PI 3 kinase) , AKT, and eNOS [38, 39] .

Conclusion: Ghrelin had potent cardiovascular protective effects. Considering that there are amount of ghrelin receptor in cardiovascular system, ghrelin may has cardioprotective significance in physiological and pathophysiological conditions. Exogenous administration with ghrelin may serve as a novel therapeutic strategy for ischemic cardiovascular diseases.

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