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Effect of ghrelin on septic shock in rats¹

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KEY WORDS septic shock; hemodynamics; ghrelin

ABSTRACT

AIM: To study the role of ghrelin in the late stage of septic shock in rats. **METHODS:** The rat model of septic shock was made by caecal ligation and perforation. At the time of operation ghrelin 10 nmol/kg was infused through femoral vein followed by a sc injection at 8 h after operation. Hemodynamic parameters including heart rate (HR), mean arterial blood pressure (MABP), $LVdp/dt_{max}$ and left ventricular end-diastolic pressure (LVEDP) in survival rats were measured at 18 h after surgery. Plasma glucose and lactate concentrations, plasma ghrelin level and myocardial ATP content were assayed. The mortality rate in rats with septic shock was also observed. **RESULTS:** Compared to that of septic shock group, MABP of rats in ghrelin-treated group increased by 33 % ($P<0.01$). The values of $+LVdp/dt_{max}$ and $-LVdp/dt_{max}$ increased by 27 % and 33 %, respectively ($P<0.01$), but LVEDP decreased by 33 % ($P<0.01$). The plasma glucose concentration and myocardial ATP content increased by 53 % and 22 %, respectively, but plasma lactate concentration decreased by 40 % in ghrelin-treated rats ($P<0.01$). The plasma ghrelin level in rats with septic shock was 51 % higher than that of rats in sham group, and was negatively correlated with MABP and blood glucose concentration ($r=-0.721$ and -0.811 , respectively, $P<0.01$). The mortality rates were 47 % (9/19) in rats with septic shock and 25 % (3/12) in rats of ghrelin-treated group, respectively. **CONCLUSION:** Treatment with ghrelin could correct partly the abnormalities of hemodynamics and metabolic disturbance in septic shock of rats.

INTRODUCTION

Ghrelin, a natural ligand of G-protein coupled with growth hormone secretagogue receptor, was purified from rat stomach in 1999^[1]. Ghrelin regulates the neuropeptide Y expressed in arcuate nucleus of hypothalamus

and promotes hypophysis to secrete growth hormone, which possesses important role in energy balance regulation^[2]. Recently, ghrelin receptors have been detected in cardiovascular tissue^[3], indicating that ghrelin may play an important regulating role in peripheral tissue other than in central nervous system. The effects of ghrelin on cardiovascular function and metabolism were paid close attention^[4-6]. The late stage of septic shock was accompanied with critical cardiovascular dysfunction^[7]. However, there has not been any report on the change in ghrelin and its effects on cardiovascular function in septic shock. The purpose of the present study was to observe the changes in plasma

¹ Project supported by the National Natural Science Foundation of China, No 39730220, and the Major State Basic Research Development Program of People's Republic of China, No G2000056905.

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Received 2002-01-11

Accepted 2002-08-05

ghrelin level, and the effects of exogenous ghrelin administration on hemodynamics and metabolism using the model of septic shock in rat made by caecal ligation and perforation.

MATERIALS AND METHODS

Reagents Glucose oxidase kit, lactate kit, and ATP assay kit were all purchased from Sigma Company. Synthetic ghrelin of rat and ghrelin radioimmunoassay kit (Lot number 416753) were produced from Phoenix Pharmaceuticals, USA. The other reagents were of analytical purity.

Experimental instruments Sep-Pak C18 cartridge was the product of Milford (MA, USA). PE-50 cannula was produced by Intramedic (NY, USA). Pressure transducer was produced by Gould P231D, USA. Physiological polygraph was the product of San ei 2G66, Japan.

Animals Thirty-seven male Sprague-Dawley rats, weighing (260 ± 10) g, were obtained from the Experimental Animal Center of Peking University (Clean grade, Certificate No SCXK 11-00-0008). The rats were randomly divided into septic shock group ($n=19$), ghrelin-treated group ($n=12$), and sham group ($n=6$).

Preparation of sepsis model Septic shock was induced by caecal ligation and perforation with the modified methods as described previously^[7,8]. Briefly, rats were fasted overnight but free access to water. After laparotomy was performed under halothane slight anesthesia, the cecum was ligated just distally to the ileocaecal valve and punctured twice with a 18-gauge needle. The cecum was then returned into the peritoneal cavity, and the abdomen was closed in two layers. Rats were injected with normal saline 10 mL/kg through femoral vein at the moment of surgical completion, and with sc injection of 40 mL/kg of normal saline again at 8 h after surgery. The operation for rats in ghrelin-treated group was as same as that of the septic group, except that the saline for injection contained ghrelin 10 nmol/kg. Laparotomy was performed to rats in sham group, but their cecum was neither ligated nor punctured. All rats were fasted but free access to water after operation. Several hemodynamic parameters were measured in survival rats at 18 h after surgery. The heparin-anticoagulated blood was collected from rat artery and heart was removed for further biochemical assays.

Measurement of hemodynamic parameters^[9]

Hemodynamic parameters were determined with polygraph via femoral artery and intraventricular cannula. The rats were anesthetized by ip injection of urethane (1 g/kg). Two PE-50 tubings were inserted into left femoral artery and left carotid artery, respectively. The latter was further inserted into the left ventricle. All catheters were filled with 0.9 % NaCl containing 10 kU/L of heparin. The ventricular and arterial catheters were separately connected with pressure transducer. Heart rate (HR), mean arterial blood pressure (MABP), $+LVdp/dt_{max}$, $-LVdp/dt_{max}$, and left ventricular end-diastolic pressure (LVEDP) were recorded on a microcomputer-controlled physiological polygraph at 20 min after inserting catheters.

Assay for concentrations of plasma lactate, plasma glucose, and myocardial ATP content Plasma was prepared by centrifugation of whole blood at $2900 \times g$, $4^{\circ}C$ for 10 min. Plasma glucose was determined by glucose oxidase method, and plasma lactate was assayed by colorimetric method. Removed heart was promptly frozen by a freeze-clamping with aluminum clamps precooled in liquid nitrogen, and the tissue was pulverized with a pestle and mortar precooled in liquid nitrogen. Myocardial ATP content was assayed spectrophotometrically using glucose-6-phosphate dehydrogenase and hexokinase methods.

Radioimmunoassay for plasma ghrelin level Blood samples were collected into tubes containing 1 g/L of ethylene diamine tetraacetate-2 Na and 500 MIU/L of aprotinin, from which the plasma was prepared by centrifugation at $2900 \times g$, $4^{\circ}C$ for 10 min and stored at $-80^{\circ}C$ until used. Plasma was acidified with 60 μ L of acetic acid 1 mol/L and diluted with 5 mL of saline solution and then loaded onto a Sep-Pak C18 cartridge equilibrated with saline. After the cartridge was washed with 2.5 mL of saline and 10 % acetonitrile in 0.1 % trifluoroacetic acid (TFA), the absorbed material was eluted with 2 mL of 50 % acetonitrile in 0.1 % TFA. The extracted material was lyophilized and subjected to radioimmunoassay for ghrelin. The sensitive IC_{50} was 23.5 pg per tube, and binding was 35 % for this assay. There was 100 % of cross-reactivity between rat and human ghrelin, but no cross-reactivity for ghrelin to vasoactive intestinal polypeptide (VIP) or galanin.

Statistical analysis Data were expressed as mean \pm SD. Comparisons between more than two groups were made by analysis of variance (one-way ANOVA) followed by Student-Newman-Keuls test. χ^2 test was used for comparisons of mortality rate among groups.

$P < 0.05$ was considered statistically significant.

RESULTS

Effects of ghrelin on the mortality rate in rats with septic shock All six rats in sham group were alive during the experiment period. About 47 % (9/19) of rats in septic group died within 18 h after operation. However, the mortality rate was only 25 % (3/12) in ghrelin-treated rats ($P = 0.144$).

Effects of ghrelin on the hemodynamic parameters in rats with septic shock Compared with sham group, the septic rats had serious hypotension and bradycardia. Cardiac systolic and diastolic function ($+LVdp/dt_{max}$ and $-LVdp/dt_{max}$) was impaired markedly (Tab 1), and LVEDP increased significantly ($P < 0.01$). However, compared with septic shock rats, MABP and HR in ghrelin-treated rats elevated by 33 % and 20 %, respectively ($P < 0.01$). And the values of $+LVdp/dt_{max}$ and $-LVdp/dt_{max}$ increased by 27 % and 33 %, respectively ($P < 0.01$), and LVEDP decreased by 33 % ($P < 0.01$).

Effects of ghrelin on the concentrations of plasma glucose, plasma lactate, and myocardial ATP in rats with septic shock As shown in Tab 2, the septic rats existed serious hypoglycemia and hyperlactacidemia, and the myocardial energy production significantly decreased. Compared with septic shock group, ghrelin treatment significantly elevated the concentration of plasma glucose and myocardial ATP level by 54 % and 22 % ($P < 0.01$), respectively, but the plasma lactate concentration decreased by 40 % ($P < 0.01$).

Plasma ghrelin level In rats with septic shock, the plasma ghrelin level was 51 % higher than sham controls ($P < 0.01$, Fig 1). The plasma ghrelin level was negatively correlated with MABP and blood glucose concentration, respectively (correlation coefficients were -0.721 and -0.811, respectively, $P < 0.01$). But it

Tab 2. Effects of ghrelin on the concentrations of plasma glucose (mmol/L), plasma lactate (mmol/L) and myocardial ATP (mmol/g wet wt) in rats with septic shock. Mean \pm SD. ^c $P < 0.01$ vs sham group. ^f $P < 0.01$ vs septic shock group.

	Plasma glucose	Plasma lactate	Myocardial ATP
Sham group ($n=6$)	6.6 \pm 0.8	1.1 \pm 0.2	4.4 \pm 0.4
Septic shock group ($n=10$)	2.7 \pm 0.4 ^c	7.2 \pm 0.9 ^c	2.6 \pm 0.4 ^c
Ghrelin-treated group ($n=9$)	4.2 \pm 0.6 ^{cf}	4.4 \pm 1.0 ^{cf}	3.2 \pm 0.4 ^{cf}

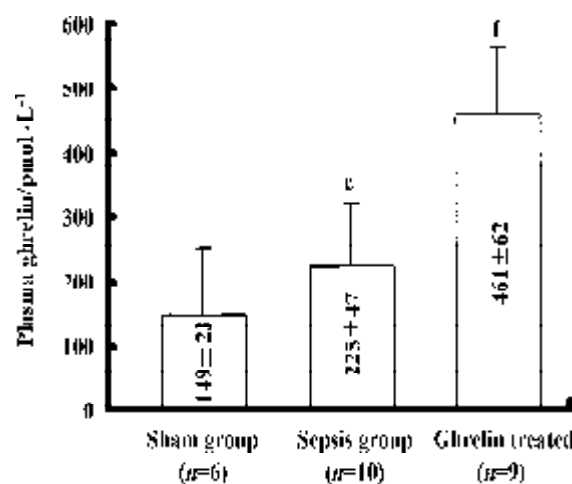


Fig 1. Plasma ghrelin level in rats with septic shock. Mean \pm SD. ^c $P < 0.01$ vs sham group. ^f $P < 0.01$ vs sepsis group.

was not correlated with blood lactate concentration ($r = 0.498$, $P > 0.05$).

DISCUSSION

Ghrelin, an endogenous natural ligand for growth hormone secretagogue (GHS) receptor originally isolated from stomach^[1], regulates pituitary growth hor-

Tab 1. Effects of ghrelin on the hemodynamic parameters in rats with septic shock. Mean \pm SD. ^c $P < 0.01$ vs sham group. ^f $P < 0.01$ vs septic shock group.

	MABP	HR	LVEDP	$+LVdp/dt_{max}$	$-LVdp/dt_{max}$
Sham group ($n=6$)	90 \pm 4	401 \pm 19	5 \pm 2	4727 \pm 481	4260 \pm 432
Septic shock group ($n=10$)	51 \pm 10 ^c	268 \pm 32 ^c	15 \pm 3 ^c	2946 \pm 273 ^c	2390 \pm 240 ^c
Ghrelin-treated group ($n=9$)	68 \pm 9 ^{cf}	321 \pm 38 ^{cf}	10 \pm 3 ^{cf}	3726 \pm 365 ^{cf}	3169 \pm 238 ^{cf}

MABP: mean artery blood pressure/mmHg; HR: heart rate/beats per min; LVEDP: left ventricular end-diastolic pressure/mmHg; $LVdp/dt_{max}$: left ventricular dp/dt maximum/mmHg·s⁻¹.

mone (GH) secretion along with neuropeptide Y, GH-releasing hormone and somatostatin^[2]. GHS receptors were found not only in pituitary and hypophysis, but also in peripheral tissues such as the myocardium, aorta, pulmonary, coronary artery, and vein^[3], suggesting that ghrelin might exert cardiovascular effects by GH-independent mechanisms. Nagaya *et al*^[4] reported that human ghrelin (10 µg/kg) elicited a potent, long-lasting GH release and had beneficial hemodynamic effects via reducing cardiac afterload and increasing cardiac output in six healthy male volunteers. Rats with chronic heart failure (CHF) were administered rat ghrelin (100 µg/kg, sc, bid) for 3 weeks, and the results showed that ghrelin significantly elevated cardiac output, left ventricular fractional shortening and $LVdp/dt_{max}$, and inhibited left ventricular enlargement in rats with CHF^[6]. These data suggested that ghrelin might protect heart directly in an independent GH manner.

Up to now, the pathogenesis of septic shock has not been fully understood. Although great progress has been made by antibiotics and therapeutics with vascular function regulators, high mortality rate is still the most important issue of sepsis, particularly in cardiac function impairment and multiple system organ failure (MSOF)^[7]. Therefore, it has important theoretical implication and clinically practical value to clarify the regulating mechanism and to explore the new therapeutics of septic shock. In this study, we used septic shock model prepared by rat caecal ligation and perforation. The results showed a serious hypotension and heart failure (+ $LVdp/dt_{max}$ and - $LVdp/dt_{max}$ decreased, LVEDP elevated and myocardial ATP content decreased) and critical metabolic disturbance (serious hypoglycemia and hyperlactacidemia) in septic shock, mimicking with the reported late stage of septic shock^[7]. Injection of ghrelin 10 nmol/kg twice significantly elevated blood pressure and improved heart function. Treatment with ghrelin also significantly increased plasma glucose concentration, decreased plasma lactate concentration and recovered myocardial ATP content in rats with septic shock, which suggested that ghrelin could correct the metabolic disturbance. Furthermore, ghrelin treatment decreased the mortality rate in rats with sepsis. But the change was not significant between septic shock group and ghrelin-treated group, which might result from insufficient sample numbers. The above findings suggested that ghrelin exerted markedly curative effects on septic shock.

However, the latent mechanisms by which ghrelin

improves cardiovascular function and metabolic disturbance are still unclear. The GH level in circulation elevated after ghrelin injection both in animal and in human^[4-6]. GH possesses potent myocardial inotropic effects, and has significantly therapeutic effects on serious heart failure and ischemic heart disease^[10,11]. O' Lenny *et al*^[12] reported that GH binding protein (GHBP) increased at 24 h and remained elevated 72 h following rat caecal ligation and perforation, and returned to baseline by 96 h. This result suggested that 'GH resistance' was associated with an increased GHBP in sepsis. Furthermore, GH administration could markedly elevate survival rate of burned septic shock in BALB/C mice^[13]. However, further studies need to be carried out to identify whether ghrelin may result in an improvement of cardiovascular function via stimulating endogenous GH release in septic shock. Since ghrelin receptors were detected in cardiovascular tissues^[3], ghrelin seemed likely to possess direct effects on cardiovascular system. Hexarelin, another endogenous natural ligand of GSH-R, could protect heart directly in a GH-independent manner on both isolated myocardial cells and myocardial ischemia-reperfusion model^[14-16].

In this study, we also observed that plasma ghrelin level elevated in rats with septic shock, and was negatively correlated with MABP and plasma glucose concentration. The plasma ghrelin level elevated further after exogenous administration of ghrelin, and so did the MABP and plasma glucose concentration. In cachexia of starved or heart failure rats, the plasma ghrelin level elevated compensately to inhibit catabolism and enhance anabolism, leading to an energy balance in a certain pathophysiological states^[4-6]. Therefore, an elevation of plasma ghrelin level may be considered as an adaptive protective response to septic shock. The fact that exogenous administration of ghrelin partially corrected the myocardial dysfunction and metabolic disturbance in septic shock indicated that ghrelin might be one of the endogenous anti-shock factors, and play an important regulatory role in cardiovascular function in septic shock.

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