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Chronic central ghrelin infusion reduces blood pressure and heart rate despite increasing appetite and promoting weight gain in normotensive and hypertensive rats

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ABSTRACT

Acute studies showed that ghrelin acts on the central nervous system (CNS) to reduce blood pressure (BP), heart rate (HR) and sympathetic activity. However, the long-term CNS cardiovascular actions of ghrelin are still unclear. We tested whether chronic intracerebroventricular (ICV) infusion of ghrelin causes sustained reductions in BP, HR and whether it alters baroreceptor sensitivity (BRS) and autonomic input to the heart. A cannula was placed in the lateral ventricle of male Sprague–Dawley (SD) rats for ICV infusions via osmotic minipump (0.5 μl/h). BP and HR were measured 24-h/day by telemetry. After 5 days of control measurements, ghrelin (0.21 nmol/h) or saline vehicle were infused ICV for 10 days followed by a 5-day post-treatment period. Chronic ICV ghrelin infusion increased food intake (22 ± 3 to 26 ± 1 g/day) leading to ~50 g body weight gain. BP fell slightly during ghrelin infusion while HR decreased by ~26 bpm. In control animals BP and HR increased modestly. ICV Ghrelin infusion caused a 50% reduction in sympathetic tone to the heart but did not alter BRS. We also tested if the depressor responses to ICV ghrelin infusion were enhanced in spontaneously hypertensive rats (SHR) due to their high basal sympathetic tone. However, we observed similar BP and HR responses compared to normotensive rats. These results indicate that ghrelin, acting via direct actions on the CNS, has a sustained effect to lower HR and a modest impact to reduce BP in normotensive and hypertensive animals despite increasing appetite and body weight.

1. Introduction

Ghrelin, an acylated 28 amino acid peptide secreted mainly by the oxyntic glands of the stomach, plays an important role in the regulation of pituitary function and in the physiological daily patterns of hunger and meal initiation [3,5,9]. Ghrelin administration promotes increased appetite and adiposity [14,15], whereas ghrelin receptor deficient mice, although exhibiting a normal body weight when fed a standard chow, are resistant to high fat-induced obesity [19,20]. The actions of ghrelin to control appetite and body weight homeostasis appear to be mediated mainly by activation of ghrelin receptors in orexigenic neurons that express neuropeptide Y (NPY) and agouti-related peptide (AGRP) located in the hypothalamus [15]. These neurons represent a important site for integration of a variety of signals originating from the periphery that arrive in the central nervous system (CNS) to inform the brain about the body's energy status and to initiate a cascade of events to maintain whole-body energy homeostasis [6].

These neurons, however, are not only important in body weight and appetite regulation but have also been demonstrated to participate in cardiovascular regulation. For instance, chronic intracerebroventricular (ICV) infusion of NPY causes bradycardia [2], while chronic AGRP treatment reduces blood pressure (BP) and heart rate (HR) despite promoting weight gain which is normally associated with increases in BP and HR [17]. Previous acute studies have shown that ghrelin infusion in healthy subjects and patients with congestive heart failure also elicits a hypotensive response including reductions in BP and HR [12,13]. To test whether the acute hypotensive effects of ghrelin are mediated by its direct actions on the CNS, Matsumura et al. showed that acute intracerebroventricular (ICV) ghrelin administration in conscious rabbits caused a dose-dependent reduction in BP, HR and renal sympathetic nerve activity (SNA) [11]. In addition, ghrelin receptors have been found to be expressed in pre-ganglionic sympathetic neurons [4] and ghrelin microinjection into the nucleus of the tractus solitarius (NTS) elicits hypotensive responses [10]. These observations reinforce the notion that ghrelin may play a role in the regulation of SNA, and suggest that ghrelin may also contribute to the day-to-day regulation of cardiovascular function.

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Despite strong evidence from these acute studies that ghrelin may exert an important effect on arterial pressure and other cardiovascular parameters, to our knowledge no study has investigated whether ghrelin is capable of producing sustained effects on cardiovascular function to cause long-term decreases in BP and HR via its actions on the CNS. Therefore, this study was designed to determine whether chronic CNS administration of ghrelin in normal Sprague–Dawley (SD) rats leads to sustained reductions in BP and HR, despite promoting weight gain and increasing food intake. In addition, we also investigated if chronic CNS ghrelin infusion alters spontaneous baroreceptor sensitivity (BRS) and autonomic input to the heart of SD rats, and whether spontaneously hypertensive rats (SHR) exhibit exacerbated response to the CNS depressor effects of ghrelin.

2. Methods

2.1. Animal surgeries

The experimental procedures and protocols of this study conform to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center.

2.1.1. Blood pressure telemetry probe and femoral vein catheter implantation

Male Sprague–Dawley rats ($n = 25$) (Harlan, Indianapolis, IN) and spontaneously hypertensive rats (SHR, $n = 6$, Taconic Farms, Hudson, NY) weighing 275–330 g were anesthetized with 50 mg/kg sodium pentobarbital (Nembutal) and atropine sulfate (0.1 mg/kg) was administered to prevent excess airway secretions. Using aseptic techniques, a laparotomy was performed and the catheter of the pressure telemetry transmitter (Model TA11PAC40, Data Sciences International, MN) was inserted into the abdominal aorta, distal to the kidneys. The catheter was fixed in the aorta with a small drop of cyanoacrylate adhesive and the transmitter was secured to the abdominal wall by sutures. Mean daily 24-hour BP data was derived from the average BP measured in bursts of 10 s every 10 min using the software (Dataquest 4.0) provided by the manufacturer. A venous catheter was also inserted in to the left femoral vein for intravenous infusion. The catheter was exteriorized in the scapular region through a subcutaneously implanted stainless steel button. After recovery from anesthesia, rats were housed in metabolic cages for determination of daily food intake. The venous catheter was connected to an infusion swivel (Instech).

2.1.2. Intracerebroventricular cannulation (ICV)

Immediately after telemetry probe and venous catheter implantation, a stainless steel cannula (26 gauge, 10 mm long) was implanted into the right lateral cerebral ventricle using the coordinates as previously described [4]. The guide cannula was anchored in place with 2 stainless steel machine screws, a metal cap, and dental acrylic, and a stylet was inserted to seal the cannula until use. During stereotaxic manipulation, anesthesia was maintained with 1.5–0.5% isofluorane. Several days after recovery from surgery, accuracy of the cannula placement was tested by measuring the dipsogenic response (immediate drinking of at least 5 ml of water in 10 min) to an ICV injection of 100 ng of angiotensin II. After the experiment, the animals were killed and the brains removed and sectioned to confirm the placement of the cannula.

Immediately after surgery, rats were housed individually in metabolic cages for determination of daily food consumption. Rats were provided a normal sodium diet (0.5 mmol sodium/g food, Harlan Teklad, WI). The rats were allowed to recover for 8–10 days before control measurements were initiated and then we began monitoring mean arterial pressure (MAP) and heart rate (HR) continuously 24 h/day using the telemetry data acquisition system (Data Science International, MN).

Table 1
Responses to chronic ICV ghrelin or vehicle infusion on blood glucose, insulin and leptin levels in Sprague–Dawley rats.

Groups	Blood glucose (mg/100 mL)	Insulin (ng/mL)	Leptin (ng/mL)
Ghrelin ($n = 4$)			
Control	90 ± 4	1.0 ± 0.2	0.5 ± 0.1
Treatment	84 ± 6	1.5 ± 0.3*	3.1 ± 1.0*
Vehicle ($n = 4$)			
Control	94 ± 3	0.8 ± 0.2	1.0 ± 0.3
Treatment	88 ± 2	0.7 ± 0.2**	1.4 ± 0.5**

Values represent Mean ± SEM.
* $p < 0.05$ vs. control period within same group (paired t test).
** $p < 0.05$ vs. ghrelin treatment (unpaired t test).

2.2. Experimental protocol

MAP, HR and food intake were recorded daily. After a 5-day control period, ghrelin (0.21 nmol/h, 0.5 μl/h, $n = 7$, AnaSpec Laboratories, CA) or vehicle (0.9% saline, $n = 8$) was infused ICV for 10 days via osmotic minipump (model 2002, Durect Corp., CA). The minipump was implanted subcutaneously in the scapular region and connected to the ICV cannula using a tygon tubing (Cole Parmer). The rate of ghrelin infusion was based on a previous study showing that a comparable dose scaled for rabbits (weighing 2.5–2.7 kg) caused marked acute decreases in MAP and HR that peaked approximately 40–50 min post injection [11]. On the last day of ghrelin or saline ICV infusion, the cannula connecting the minipump with the ICV cannula was severed to stop the infusion and the rats followed for an additional 5-day post-treatment period.

In separate groups of animals ($n = 4$/group), blood samples (250 μl) were collected after a 6-hour fast on day 5 of control period and on day 10 of ghrelin or vehicle infusion for determination of insulin, leptin and blood glucose levels. Blood glucose levels were measured using glucose strips (Reli On), insulin and leptin concentrations were determined using rat ELISA kits from Crystal Chem and R&D Systems, respectively. The intra and inter-assay sensitivity of these commercially available kits are <10% for intra and inter-assay for the rat insulin ELISA kit and <5% and <7% for intra and inter-assay sensitivity for the rat leptin ELISA kit.

2.2.1. Air-jet stress test

To determine whether chronic ICV ghrelin infusion alters the BP response to an acute pressor stress, rats from both groups ($n = 5$/group) were subjected to an acute air-jet stress test once during the control period and again on day 9 of ghrelin ICV infusion between 10:00 am and 11:00 am. Briefly, after a 30-minute baseline period an air-jet stress test consisting of 2-second pulses of air jet delivery every 10 s during 5 consecutive minutes aimed at the forehead of the rats at an approximately distance of 15 cm using a 14 gauge needle opening at the front of the tube connected to compressed air. BP responses during the air-jet stress were calculated as the changes compared to baseline period (average of the last 10 min of baseline measurements before air-jet stress was initiated). The rats were kept in their home cages during the air-jet stress test.

2.2.2. Baroreflex sensitivity (BRS)

Spontaneous baroreflex sensitivity (BRS) was determined once during the control period and on the last day of ghrelin or saline ICV infusion. BRS was calculated using continuous BP and HR recordings (120 min at 1000 Hz, between 2:00 pm and 4:00 pm)