Published in final edited form as:

Peptides. 2013 April ; 42: 35–42. doi:10.1016/j.peptides.2013.02.003.

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

John N. Freeman, Jussara M. do Carmo, Ahmad H. Adi, and Alexandre A. da Silva

Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS

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 μ l/hr). BP and HR were measured 24-hr/day by telemetry. After 5 days of control measurements, ghrelin (0.21 nmol/hr) 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 was 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.

Keywords

Ghrelin; heart rate; baroreceptor function; blood pressure; food intake; leptin

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,

© 2013 Elsevier Inc. All rights reserved.

Corresponding Author: Alexandre A. da Silva, Ph.D., Department of Physiology and Biophysics University of Mississippi Medical Center, 2500 North State St., Room: G263, Jackson, MS 39216-4505, Phone: (601) 984-1826, Fax: (601) 984-1833, asilva@umc.edu.

8. CONFLICT OF INTEREST/DISCLOSURES

None.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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 an 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.

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-hr BP data was derived from the average BP measured in bursts of 10 seconds every 10 minutes 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% to 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 minutes) 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 hrs/day using the telemetry data acquisition system (Data Science International, MN).

2.2 Experimental Protocol

MAP, HR and food intake were recorded daily. After a 5-day control period, ghrelin (0.21 nmol/hr, 0.5 μ l/hr, 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 to 2.7 kg) caused marked acute decreases in MAP and HR that peaked approximately 40–50 minutes 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 μ l) 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 seconds 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 minutes 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) by the sequence method based on quantification of sequences of at least three heart beats in which systolic arterial pressure (SAP) consecutively increases (up sequence) or decreases (down sequence) accompanied by changes in the same direction of the RR intervals (RRI's) of the subsequent beats as previously described [1]. The following criteria were used to estimate spontaneous BRS: 1) minimal RRI change - 1 ms; 2) minimal SAP changes - 1 mmHg; c) minimal number of beats - 3 consecutive beats; 4) minimal correlation coefficient - 0.85. Spontaneous BRS was calculated in the time (sequences up, down and total) and frequency (low-frequency (LF) and high-frequency (HF) α indices) domains.

2.2.3 Autonomic input to the heart—To determine whether chronic ICV ghrelin treatment alters sympathetic and/or parasympathetic input to the heart, cardiac sympathetic and parasympathetic tone were evaluated in a separate groups of animals (n=5/group) once during control period and on the last day of ICV ghrelin infusion between 4:00 pm and 4:45 pm. Briefly, under resting conditions the chronotropic effects of full-blocking doses of propranolol (4 mg/kg), a β -adrenergic receptor antagonist, and atropine (2 mg/kg), a muscarinic receptor blocker at a volume of 0.2 ml per intravenous injection. Propranolol was injected first followed 15 minutes later by an injection of atropine.

2.3 Statistical analysis

Data are reported as mean $\pm$ SEM. Food intake, body weight, MAP, HR and spontaneous BRS values between control, experimental and post-treatment periods were compared by one-way ANOVA for repeated measures followed by Tukey's post hoc test. Comparisons between different groups were done by unpaired *t* test or two-way ANOVA when appropriate. Statistical significance was accepted at a level of $P < 0.05$.

3. RESULTS

3.1 Chronic ICV ghrelin infusion increases food intake and promotes weight gain in SD rats

Chronic ICV ghrelin infusion caused a modest (~20%) increase in daily food intake (Figure 1A). This modest increase in food intake, however, caused a significant increase in the net cumulative food intake over the 10 days of treatment (Figure 1B) which was associated with significant weight gain (Figure 1C). When ghrelin infusion was stopped we observed a marked rebound effect where food intake during the 5-day post-treatment period was less than during the control period (Figure 1A), and resulted in a significant net food intake deficit of approximately 30 g (Figure 1B). In contrast, animals that were infused with the saline vehicle exhibited an initial reduction in food intake during the first 3 days of treatment, likely due to the stress of surgical implantation of the minipump, which resulted in a modest net food intake deficit and a small weight loss (Figures 1A, 1B and 1C, respectively). When saline infusion was stopped food intake did not change significantly and there was no change in cumulative food intake during the recovery period when compared to

the control period (Figure 1B). These control animals exhibited normal weight gain during the post-treatment period (Figure 1C).

The weight caused by ghrelin infusion was also associated with increased circulating levels of insulin (~50%) and leptin (6-fold), whereas no significant change was observed in vehicle-infused rats (Table 1). Blood glucose levels were not altered by either treatment (Table 1).

3.2 Chronic ICV ghrelin infusion reduced BP, HR and BP response to acute air-jet stress test in SD rats

At baseline, BP and HR were similar between groups (98 ± 1 and 102 ± 2 mmHg and 352 ± 9 and 355 ± 7 bpm, respectively for ghrelin and vehicle groups, respectively). Chronic ghrelin infusion for 10 days caused a gradual small reduction in MAP ($p < 0.05$ in the last 2 days of treatment, Figures 2A and 2B) despite the weight gain and increased food intake which normally tends to increase arterial pressure. In addition to the small decrease in BP, ghrelin also caused a significant reduction in HR of approximately 26 bpm during the last 5 days of treatment (Figures 2C and 2D). ICV vehicle infusion caused a small initial increase in MAP and HR during the first 3–4 days of treatment which soon returned to baseline control values (Figure 2). The slight initial increase in BP and HR in vehicle-treated rats is also likely due to the stress of the minor procedure to implant the osmotic minipump. In a separate group of animals ($n=4$), the same dose of ghrelin was administered intravenously for 7 days to test if any potential spillover of ghrelin into the systemic circulation could account for its hypotensive effects, however no significant change in HR, BP or food intake was observed during the 7-day treatment period (Figure 3).

We also examined if chronic ghrelin ICV infusion altered circadian variations in BP and HR. However, the reductions in BP and HR during ghrelin infusion were of similar magnitude when analyzing diurnal and nocturnal changes in BP (day-time BP: 96 ± 2 vs. 93 ± 2 mmHg; nighttime: 99 ± 2 vs. 96 ± 2 mmHg) or HR (day-time: 316 ± 2 vs. 292 ± 3 bpm; night-time: 352 ± 3 vs. 327 ± 6 bpm, respectively for control period vs. average of the last 3 days of ICV ghrelin infusion).

In addition to reduce BP and HR, ICV ghrelin treatment also reduced the BP response to an acute stressor by approximately 20%, whereas vehicle infusion had no effect to attenuate the rise of BP during an air-jet stress test (Figure 4A).

3.3 Chronic ICV ghrelin infusion reduced sympathetic tone to the heart but did not alter spontaneous BRS in SD rats

To examine whether the marked reduction in HR during ICV ghrelin infusion was due to a reduction of sympathetic tone and/or increased parasympathetic tone to the heart we examined their individual contribution to the regulation of HR during control and ghrelin treatment in both groups. Chronic ICV ghrelin infusion for 10 days markedly reduced cardiac sympathetic tone by 50% while no significant change was observed on parasympathetic tone (Figure 4B).

Although chronic ghrelin infusion reduced HR and sympathetic tone to the heart, and caused a small decrease in MAP, it did not significantly alter spontaneous BRS (1.36 ± 0.25 vs. 1.25 ± 0.18 ms/mmHg for control compared to day 10 of ICV ghrelin infusion, respectively). Chronic ICV vehicle infusion also did not alter spontaneous BRS.

3.4 Chronic ICV ghrelin infusion caused bradycardia and modest reduction in BP in SHR

To examine ICV ghrelin infusion would produce a more pronounced effect to lower BP in a model of high baseline sympathetic tone we also infused ghrelin ICV in SHR for 7 days. Similar to its effect on normotensive SD rats, ghrelin caused a gradual marked reduction in HR (Figures 5C and 5D). However, it also led to only a modest reduction in BP of approximately 3 to 4 mmHg as observed in SD rats (Figures 5A and 5B). Thus, suggesting that ghrelin's depressor action is not affected by the degree of baseline sympathetic tone.

Ghrelin also increased appetite and promoted weight gain in SHR in a similar fashion than in SD rats (data not shown).

4. DISCUSSION

In the present study we sought to determine if chronic CNS administration of ghrelin, in addition to increasing appetite, would also cause sustained reductions in BP and HR since previous studies have demonstrated that acute ghrelin infusion into the CNS markedly reduces BP, HR and renal SNA. Our findings, however, suggest that the long-term hypotensive action of ghrelin mediated by the CNS is not as powerful as compared to ghrelin's acute effect on BP regulation. The chronic effect of ghrelin on HR regulation was comparable to the bradycardia observed during acute ghrelin infusion [11]. Despite these effects on BP and HR, ghrelin did not alter baroreflex function, suggesting that there was no impairment of mechanisms responsible for rapid buffering of fluctuations in BP. Ghrelin infusion was also associated with hyperphagia and increased body weight which may have contributed to the lesser reduction in BP in the present study compared to previous studies where ghrelin was injected acutely and BP followed for only a few minutes or hours. In addition, the chronic hypotensive effects of ghrelin were similar in normotensive and hypertensive rats, and these effects were comparable during both the active night phase as well as during the inactive day phase. This suggests that differences in baseline BP or basal sympathetic tone do not alter the response to ICV ghrelin infusion on BP regulation, and that ghrelin does not alter the normal circadian fluctuations of BP and HR.

As previously mentioned, the rapid weight gain caused by increased appetite and the associated rise in circulating insulin and leptin levels may have diminished some of ghrelin's hypotensive actions. We have not performed pair-feeding experiments to prevent weight gain and the elevations in insulin and leptin levels in ghrelin-treated rats since this approach may also result in stress-induced sympathetic activation. For example, we previously showed that the hypotensive actions of blocking the CNS melanocortin 3 and 4 receptors (MC3/4R) were not exacerbated by pair-feeding to prevent the marked hyperphagia and weight gain caused by MC3/4R blockade [7]. Another potential explanation for the weaker long-term hypotensive action ghrelin compared to its acute effect may be that ghrelin initially leads to a marked reduction in SNA to the blood vessels of various tissues resulting in peripheral vasodilation and a rapid fall in BP, whereas its ability to suppress renal SNA, which would have a major impact on long-term control of BP, may be modest resulting in only a small reduction in BP over a prolonged period of time. In fact, although ghrelin caused only a very modest reduction in BP in SD and SHR, it reduced by 20% the rise in BP during an acute pressor stress test and it also markedly reduced sympathetic tone to the heart. These observations indicate that ghrelin is capable of suppressing, at least in part, the mechanisms responsible for rapid increases in BP (most likely buffering the increase in sympathetic activity to peripheral tissues). Thus, supporting the earlier statement that ghrelin may preferentially control sympathetic activity to blood vessels and the heart while not altering sympathetic activity to the kidneys. Moreover, vehicle-treated rats exhibited the normal stress response to implantation of osmotic minipumps characterized by a transient reduction in food intake and slight increase in BP and HR, while animals treated with

ghrelin did not. This observation also supports our finding that ghrelin blunts the cardiovascular response to stress stimuli.

Contrary to its modest effect on BP, ghrelin caused a substantial reduction in HR in both SD and SHRs. This effect appears to have been due solely to suppression of sympathetic input to the heart since ghrelin reduced cardiac sympathetic tone by 50% whereas it had no impact on cardiac parasympathetic tone. Whether ghrelin reduced sympathetic input to the heart by direct actions on brainstem nuclei or indirectly via projections from the hypothalamus is unclear and deserves further investigation.

The signaling mechanisms by which ghrelin alters sympathetic activity and lowers BP and HR are still not fully understood. Ghrelin has been shown to exert its metabolic actions mainly by activating its receptors located in NPY/AGRP neurons in the hypothalamus [15]. Although chronic central NPY infusion reduces HR [2], no previous studies, to our knowledge, have demonstrated a major effect of NPY in long-term regulation of BP. Chronic central AGRP infusion however, causes modest but sustained reductions in BP and this occurs despite marked hyperphagia and rapid weight gain [17]. In addition, we have also previously shown that chronic activation of MC3/4R is associated with increased SNA and elevation in BP and HR [8], and that an intact MC4R is necessary for obesity to be associated with increased BP [18]. Taken together, these observations suggest that one potential mechanism by which ghrelin alters BP and HR is via stimulation of NPY/AGRP neurons and consequent inhibition of POMC/CART neurons, ultimately resulting in reduced activation of MC3/4R. Another potential mechanisms contributing to the hypotensive effects of ghrelin may be the stimulation of growth hormone release which have been shown to acutely reduce BP (16). The role of growth hormone, NPY, AGRP or MC3/4R in mediating the effects of ghrelin on BP and HR regulation, however, is beyond the scope of the present study and additional studies are needed to test these possibilities. Ghrelin, as expected, also increased food intake and body weight. This increase in food intake during the 10-day treatment period was followed by a rapid reduction of appetite to levels below those observed during control period before beginning the infusion of ghrelin. This suggests that ghrelin also triggered the activation of compensatory factors that oppose ghrelin's orexigenic effects which, when ghrelin infusion was stopped, reduced food intake and promoted weight loss.

5. CONCLUSIONS

Our results indicate that despite increasing food intake and promoting rapid weight gain chronic central administration of ghrelin for 10 days caused only a modest reduction in BP and a more pronounced fall in HR without affecting spontaneous BRS. These observations suggest that although the chronic actions of ghrelin on BP regulation do not recapitulate its strong hypotensive acute effects, ghrelin may still play a role in the long-term control of HR and BP which may have been diminished by its metabolic effects that increase appetite and promote weight gain. Thus, reduction in circulating ghrelin levels reported in obese individuals may also contribute, at least in part, to the overactivity of sympathetic nervous system in obesity. The precise mechanisms by which ghrelin regulates sympathetic activity by its direct actions on the CNS and whether it alters CNS sensitivity to other peptides/hormones that also participate in the regulation of sympathetic activity are still unclear and represent an important area for further investigation.

Acknowledgments

We thank Dr. John E. Hall for his suggestions and careful review of this manuscript and Haiyan Zhang for the measurement of insulin and leptin levels.

7. SOURCES OF FUNDING

The authors' research was supported by a National Heart, Lung and Blood Institute grant PO1HL-51971 and by a Scientist Development Grant from the American Heart Association to Alexandre A. da Silva and Jussara M. do Carmo.

References

1. Baudrie V, Laude D, Elghozi JL. Optimal frequency ranges for extracting information on cardiovascular autonomic control from blood pressure and pulse interval spectrograms in mice. *Am J Physiol Regul Integr Comp Physiol*. 2007; 292:R904–R912. [PubMed: 17038438]
2. Correia ML, Morgan DA, Sivitz WI, Mark AL, Haynes WG. Hemodynamic consequences of neuropeptide Y-induced obesity. *Am J Hypertens*. 2002; 15:137–142. [PubMed: 11865825]
3. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes*. 2001; 50:1714–1719. [PubMed: 11473029]
4. Ferens DM, Yin L, Bron R, Hunne B, Ohashi-Doi K, Kitchener PD, Sanger GJ, Witherington J, Shimizu Y, Furness JB. Functional and in situ hybridization evidence that preganglionic sympathetic vasoconstrictor neurons express ghrelin receptors. *Neuroscience*. 2010; 166:671–679. [PubMed: 20060438]
5. Frecka JM, Mattes RD. Possible entrainment of ghrelin to habitual meal patterns in humans. *Am J Physiol Gastrointest Liver Physiol*. 2008; 294:G699–G707. [PubMed: 18187517]
6. Hillebrand JJ, de Wied D, Adan RA. Neuropeptides, food intake and body weight regulation: a hypothalamic focus. *Peptides*. 2002; 23:2283–2306. [PubMed: 12535710]
7. Kuo JJ, Silva AA, Hall JE. Hypothalamic melanocortin receptors and chronic regulation of arterial pressure and renal function. *Hypertension*. 2003; 41:768–774. [PubMed: 12623994]
8. Kuo JJ, da Silva AA, Tallam LS, Hall JE. Role of adrenergic activity in pressor responses to chronic melanocortin receptor activation. *Hypertension*. 2004; 43:370–375. [PubMed: 14707160]
9. Lanfranco F, Motta G, Baldi M, Gasco V, Grotoli S, Benso A, Broglio F, Ghigo E. Ghrelin and anterior pituitary function. *Front Horm Res*. 2010; 38:206–211. [PubMed: 20616513]
10. Lin Y, Matsumura K, Fukuhara M, Kagiya S, Fujii K, Iida M. Ghrelin acts at the nucleus of the solitary tract to decrease arterial pressure in rats. *Hypertension*. 2004; 43:977–982. [PubMed: 14993197]
11. Matsumura K, Tsuchihashi T, Fujii K, Abe I, Iida M. Central ghrelin modulates sympathetic activity in conscious rabbits. *Hypertension*. 2002; 40:694–699. [PubMed: 12411464]
12. Nagaya N, Kojima M, Uematsu M, Yamagishi M, Hosoda H, Oya H, Hayashi Y, Kangawa K. Hemodynamic and hormonal effects of human ghrelin in healthy volunteers. *Am J Physiol Regul Integr Comp Physiol*. 2001; 280:R1483–R1487. [PubMed: 11294772]
13. Nagaya N, Miyatake K, Uematsu M, Oya H, Shimizu W, Hosoda H, Kojima M, Nakanishi N, Mori H, Kangawa K. Hemodynamic, renal, and hormonal effects of ghrelin infusion in patients with chronic heart failure. *J Clin Endocrinol Metab*. 2001; 86:5854–5859. [PubMed: 11739451]
14. Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S. A role for ghrelin in the central regulation of feeding. *Nature*. 2001; 409:194–198. [PubMed: 11196643]
15. Nogueiras R, Williams LM, Dieguez C. Ghrelin: new molecular pathways modulating appetite and adiposity. *Obes Facts*. 2010; 3:285–292. [PubMed: 20975294]
16. Nystrom HC, Klintland N, Caidahl K, Bergstrom G, Wickman A. Short-term administration of growth hormone (GH) lowers blood pressure by activating eNOS/nitric oxide (NO)-pathway in male hypophysectomized (Hx) rats. *BMC Physiol*. 2005; 5:17. [PubMed: 16271154]
17. Tallam LS, Kuo JJ, da Silva AA, Hall JE. Cardiovascular, renal, and metabolic responses to chronic central administration of agouti-related peptide. *Hypertension*. 2004; 44:853–858. [PubMed: 15545513]
18. Tallam LS, Stec DE, Willis MA, da Silva AA, Hall JE. Melanocortin-4 receptor-deficient mice are not hypertensive or salt-sensitive despite obesity, hyperinsulinemia, and hyperleptinemia. *Hypertension*. 2005; 46:326–332. [PubMed: 16027245]

19. Wortley KE, Anderson KD, Garcia K, Murray JD, Malinova L, Liu R, Moncrieffe M, Thabet K, Cox HJ, Yancopoulos GD, Wiegand SJ, Sleeman MW. Genetic deletion of ghrelin does not decrease food intake but influences metabolic fuel preference. *Proc Natl Acad Sci USA*. 2004; 101:8227–8232. [PubMed: 15148384]
20. Wortley KE, del Rincon JP, Murray JD, Garcia K, Iida K, Thorner MO, Sleeman MW. Absence of ghrelin protects against early-onset obesity. *J Clin Invest*. 2005; 115:3393–3397. [PubMed: 16322785]

Highlights

Chronic CNS ghrelin infusion increases appetite and promote weight gain.

Chronic CNS ghrelin infusion reduces BP and HR despite weight gain and hyperphagia.

Ghrelin's effect on BP and HR is associated with reduced cardiac sympathetic tone.

Figure 1.

Food intake (A), cumulative food intake (B) and body weight changes (C) in response to chronic ICV ghrelin or vehicle infusion. Data expressed as Mean±SEM. # indicates $p < 0.05$ vs. control period (1-way ANOVA repeated measures). * indicates $p < 0.05$ vs. ghrelin treatment (unpaired t test).

A)

B)

C)

D)

Figure 2.

Mean arterial pressure (A), changes in mean arterial pressure (B), heart rate (C) and changes in heart rate (D) in response to chronic ICV ghrelin or vehicle infusion. Data expressed as Mean±SEM. # indicates $p < 0.05$ vs. control period (1-way ANOVA repeated measures). * indicates $p < 0.05$ vs. ghrelin treatment (2-way ANOVA).

Figure 3. Food intake (**A**), mean arterial pressure (**B**) and heart rate (**C**) in response to chronic intravenous (IV) ghrelin infusion. Data expressed as Mean±SEM.

A)

B)

Figure 4.

Mean arterial pressure response to acute air-jet stress test (A) and cardiac sympathetic and parasympathetic tone during control period and ICV ghrelin or vehicle infusion (B). Data expressed as Mean $\pm$ SEM. # indicates $p < 0.05$ vs. control period (1-way ANOVA repeated measures). * indicates $p < 0.05$ vs. ghrelin treatment (2-way ANOVA).

A)

B)

C)

D)

Figure 5.

Mean arterial pressure (A), changes in mean arterial pressure (B), heart rate (C) and changes in heart rate (D) in response to chronic ICV ghrelin infusion in spontaneously hypertensive rats (SHR) and normotensive Sprague-Dawley (SD) rats. Data expressed as Mean±SEM. # indicates $p<0.05$ vs. control period (1-way ANOVA repeated measures). * indicates $p<0.05$ vs. SD rats (2-way ANOVA).

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.

[#] indicates p<0.05 vs. control period within same group (paired *t* test);

^{*} indicates p<0.05 vs. ghrelin treatment (unpaired *t* test).