Acute administration of acyl, but not desacyl ghrelin, decreases blood pressure in healthy humans

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Abstract

Objective: To compare the effects of acyl ghrelin (AG) and desacyl ghrelin (DAG) on blood pressure (BP), heart rate (HR) and other autonomic parameters in healthy humans and to elucidate the hormonal mechanisms through which AG could exert its cardiovascular effects.

Design: Seventeen healthy participants underwent frequent monitoring of systolic (sBP) and diastolic blood pressure (dBP), HR, respiratory rate (RR) and body surface temperature (Temp) during continuous infusion of AG, DAG, combined AG + DAG or saline control before and during an IV glucose tolerance test on 4 separate days. Plasma catecholamines, renin and aldosterone levels were also measured. Differences in outcome measures between treatment groups were assessed using mixed-model analysis.

Results: Compared to the saline control, AG and combined AG + DAG infusions decreased sBP, dBP, mean arterial blood pressure (MAP), HR and Temp. In contrast, DAG infusion did not alter BP, RR or Temp, but did decrease HR. The AG and AG + DAG infusions also raised plasma aldosterone levels compared to saline (P < 0.001) without affecting renin or catecholamine levels.

Conclusions: The decrease in BP, HR, RR and Temp with AG infusion suggests mediation through the autonomic nervous system. The lack of response to DAG suggests that these autonomic effects require activation of the ghrelin receptor.

Introduction

Ghrelin is an orexigenic peptide, synthesized primarily in the stomach (1), which is involved in multiple physiologic functions including growth hormone secretion, energy balance, glucose homeostasis and cardiovascular (CV) regulation (2). After translation but prior to secretion, intracellular ghrelin is acylated at the serine-3 residue by ghrelin O-acyltransferase (GOAT). Both acyl (AG) and desacetyl ghrelin (DAG) are released into the circulation, and under most physiologic settings, DAG is the more abundant of the two isoforms (3). Acylation is required for binding and activation of the growth hormone secretagogue receptor type 1a (GHSR1a), the only known receptor for ghrelin. Therefore, most of the biologic actions attributed to ghrelin are considered to be mediated by AG.

AG rises prior to meals and falls after eating with levels varying 2- to 3-fold between the fasting and fed states (4). As it promotes hunger and feeding behavior,
AG has been used in clinical trials as therapy for disease states associated with cachexia (5, 6). When given as a continuous infusion, AG also worsens insulin secretion, glucose tolerance and insulin sensitivity (7, 8), which is perhaps a protective mechanism against fasting hypoglycemia. Therefore, AG antagonists have been proposed as potential treatments for obesity and type 2 diabetes, although they have only been studied in preclinical trials to date (9). DAG is proposed to have potential beneficial effects on glucose tolerance in rodents (10), which has not been consistently duplicated in humans (11, 12). Despite this variability, clinical trials with DAG analogues are ongoing (13).

Recent studies also implicate a role for AG in CV function and blood pressure (BP) control, which has been highlighted in a recent review (14). The GHSR1a is expressed in the myocardium, aorta, coronary arteries, peripheral vasculature and regions of the central nervous system (CNS) involved in CV regulation including spinal cord, brainstem and midbrain autonomic preganglionic neurons (15, 16). GOAT is similarly widely expressed in human tissues including the myocardium (17). In healthy humans and those with chronic heart failure, acute ghrelin administration decreases systemic vascular resistance, whereas it increases cardiac output and stroke volume index (18, 19). In vitro, AG has direct vasodilatory effects in humans and animals through nitric oxide-dependent and -independent mechanisms (15, 20, 21). AG administered centrally also decreases blood pressure in rodents, possibly through suppression of sympathetic activity (22).

As noted previously, emerging evidence suggests that DAG may have physiologic effects that oppose AG and are independent of GHSR1a signaling (23). Similar to AG, DAG can exert endothelial-dependent and -independent vasodilatory effects on human and rodent vasculature in vitro (15, 24). In humans, lower levels of DAG are correlated with hypertension (25) and increased cardiovascular risk (26), but the direct effect of DAG on the human CV system has not been investigated prior to this study. Here, we compare the effects of AG and DAG infusions on BP and other autonomic parameters in healthy humans. We include a coadministration (AG + DAG) treatment arm to determine if DAG potentiates or antagonizes the effects of AG on the CV system. We also evaluate the effect of AG on various BP-modulating hormones to determine the mechanisms by which AG may potentiate its CV actions.

Subjects and methods

Subjects

Healthy participants between the ages of 18 and 45 years with a BMI between 18 and 29 kg/m² were recruited from...
the greater Cincinnati area to participate in a study of the effect of ghrelin on glucose tolerance (11). The data presented here were obtained contemporaneously during this study. Subjects with a history of impaired fasting glucose or diabetes mellitus, recent myocardial infarction, congestive heart failure, active liver or kidney disease, growth hormone deficiency or excess, neuroendocrine tumor, anemia or who were on medications known to alter insulin sensitivity were excluded. Women were studied during their follicular phase.

Study procedures were conducted at the Clinical and Translational Research Center (CTRC) at Cincinnati Children’s Hospital Medical Center. Study participants provided written informed consent for the study by signing a form approved by University of Cincinnati and Cincinnati Children’s Hospital Medical Center Institutional Review Boards, and all data collected and reported here are in compliance with the Declaration of Helsinki.

**Experimental protocol**

Subjects arrived at the CTRC between 07:30 and 08:00 after a 10- to 12-h fast on four occasions separated by at least five days. IV catheters were placed in veins of both forearms for blood sampling and infusion of test substances. The arm with the sampling catheter was placed in a 55°C chamber to arterialize venous blood. BP, HR, RR and Temp were monitored every 15 min using GE Dash 4000 vital sign monitors during the study procedure. Mean arterial blood pressure (MAP) was calculated using the equation MAP = ⅓ diastolic BP − ⅓ systolic BP.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Comparison of hemodynamic and autonomic parameters in response to AG and DAG.</th>
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∆LSM, differences of least squares means; AG, acyl ghrelin; BP, blood pressure; Com, combined AG+DAG; DAG, desacyl ghrelin; HR, heart rate; MAP, mean arterial pressure; RR, respiratory rate; s.e.m., standard error of the mean; Temp, body surface temperature.
Blood samples for hormone measurements were placed on ice, and plasma and serum were separated by centrifugation within 1 h and stored at −80°C until assay. Blood for AG and DAG measurements was collected directly into 4 mM 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride (AEBSF), a protease and esterase inhibitor, and 200 µL 1 M HCl was added to every milliliter of plasma.

After withdrawal of fasting blood samples, a bolus dose of synthetic human AG (CS Bio, Menlo Park, CA, USA; 0.28 µg/kg), synthetic human DAG (CS Bio; 1.1 µg/kg), combined AG + DAG or saline was given at time 0, followed by a continuous IV infusion of AG at 1.0 µg/kg/h, DAG at 4.0 µg/kg/h, combined AG (1.0 µg/kg/h) and DAG (4.0 µg/kg/h) or saline respectively, for a total of 210 min. A higher concentration of DAG was used in the study to mimic the ratio of AG:DAG found in healthy humans in the fed state (3). Based on the pharmacokinetic data from previous studies (27), ghrelin levels in the circulation were expected to reach a steady state within 30 min of ghrelin infusion.

After 30 min of peptide/saline infusion, an IV bolus of 50% dextrose solution (11.4 g/m² body surface area) was given as the commencement of an insulin-modified frequently sampled intravenous glucose tolerance test (IVGTT) (28). Subsequently, regular insulin (0.025 units/kg body wt) was infused intravenously over 5 min, starting 20 min after the glucose injection. AG and DAG levels were measured at −15, 0, 5, 15, 25, 30, 60, 90, 150 and 210 min; norepinephrine (NE) and epinephrine (Epi) levels were measured at −1, 29 and 30 min and renin and aldosterone levels were measured at −15, 29, 46, 60, 90 and 210 min.

**Statistical analysis**

Differences in baseline values of the outcome variables between treatment groups were assessed with Kruskal–Wallis test using GraphPad Prism v.6. Data were analyzed using mixed-model regression where each of the main outcomes of interest (sBP, dBP, MAP, HR, RR, Temp) was modeled as a function of treatment group, time, the interaction between time and group and the baseline

**Figure 2**

Study 1: Effects of AG and DAG on catecholamines, renin and aldosterone. Plasma aldosterone (pg/mL; Fig. 2A), renin (µIU/ml; Fig. 2B), norepinephrine (NE in pg/mL; Fig. 2C) and epinephrine (Epi in pg/mL; Fig. 2D) concentrations during a 210-min infusion of saline, acyl ghrelin (AG), desacyl ghrelin (DAG) or combined AG+DAG in 10 healthy men and women. The NE and Epi concentrations were only assayed until the 120-min time point. An intravenous glucose tolerance test (IVGTT) was also performed between 30 and 210 min after the peptide infusions began. The area under the curve (AUC) is shown for NE, Epi, renin and aldosterone.
of cardiovascular system

Fig. 2

Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Comparison</th>
<th>ΔLSM</th>
<th>s.e.m.</th>
<th>DF</th>
<th>T-Value</th>
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Table 2 Comparison of hormone concentrations in response to AG and DAG.

ΔLSM, differences of least squares means; AG, acyl ghrelin; Com, combined AG + DAG; DAG, desacyl ghrelin; DF, degrees of freedom; Epi, epinephrine; NE, norepinephrine; s.e.m., standard error of the mean.
saline infusions did not affect plasma NE and Epi levels (Fig. 2 and Table 2). AUC values for NE, Epi, renin and aldosterone after ghrelin and saline infusions are shown in Fig. 2E, F, G and H. Although neither were significant, NE and Epi AUC values show a trend to be increased by AG compared to DAG and saline.

Discussion

The role of AG in the regulation of cardiovascular function has been extensively studied, but the precise mechanism for its BP-lowering effect has not been elucidated. DAG has been reported to have GHSR-independent effects on the CV system in rodents that are largely protective (33, 34), but its effect in humans is not well understood. Our study is novel in its use of a DAG infusion to explore the direct effect of this hormone on CV tone. We also investigated the role of AG and DAG on autonomic parameters beyond BP and HR, which have not been previously studied in humans. Finally, we attempted to determine the hormonal mechanisms through which DAG and AG could exert their cardiovascular effects. Interestingly, the DAG infusion had no effect on any autonomic parameter except HR. We found that AG infusion led to a decrease in sBP, dBP and MAP, as expected, with new findings that it also lowered HR, RR and Temp. The BP-lowering effect of AG was unaffected by the coadministration of DAG. Thus, our findings imply that the effect of AG on the cardiovascular system is likely dependent on GHSR receptor activation.

Several clinical studies have demonstrated decreased arterial pressure after AG is given as IV bolus or infusion (19, 35, 36). Our study, novel in its continuous monitoring of systemic BP and HR over a 3-h infusion period, supports these previous findings as AG infusion lowered both sBP and dBP. However, the effect was modest. The average decreases in sBP and dBP from baseline were 5.2±1.7 and 5.9±1.5 mmHg respectively. We found a similarly modest, but significant decrease in HR that has been noted in some, but not all, human studies as well as rodent studies (35, 37). The discrepancies in HR effects between studies may be due to differences in AG doses or mode of administration. However, in reviewing studies that found no changes in HR despite decreased BP, we do note a nonsignificant trend of lower heart rate with AG (18, 19).

The precise mechanism(s) by which AG lowers blood pressure is not known, but the possibilities include (1) central effects on the sympathetic and parasympathetic nervous system (22, 37), (2) peripheral effects through direct action on blood vessels (15, 20) and/or (3) hormonally mediated effects on either of these systems. AG can cross the blood–brain barrier (38), and the ghrelin receptor (GHSR) is present in the autonomic preganglionic neurons (16) and the nucleus tractus solitarius (NTS) (22), a brain region that regulates autonomic outflow. Direct ghrelin injection into the NTS has been shown to decrease HR and MAP in rodents (22). Peripheral ghrelin administration can suppress cardiac sympathetic nervous activity, increase parasympathetic activity and prevent early left ventricular remodeling in rats after myocardial infarction (39). Similarly in humans, peripheral AG bolus injection decreases parameters of sympathetic nervous activity while increasing measures of parasympathetic nervous activity during Holter monitoring (35). Our findings of simultaneous decreases in BP and HR suggest that AG decreases sympathetic and/or increases parasympathetic outflow to the CV system. The negative effect of AG on catecholamine secretion observed in the current study could be due to the insufficient sample size. With the decrease in BP, we would expect a temporary rise in catecholamine levels; however, this effect may be offset by decreased sympathetic tone, which should lower catecholamines. Although increased levels of Epi have been demonstrated by studies using AG intravenous boluses (19, 36), this neutral effect of AG on Epi has been found previously in healthy humans given AG infusions at doses similar to those used in our study (7). As for the second hypothesis, although AG is also reported to act peripherally on the vasculature by decreasing tone and peripheral vascular resistance (15, 20), we would expect this effect to result in increased HR along with decreased BP instead of the decreased HR that we observed.

Not much is known about the effect of ghrelin on body temperature or respiratory drive in humans. In rodents, intracerebroventricular (ICV) injection of AG (40) and DAG (41) decreases core body temperature. This effect could be mediated through decreased brown adipose tissue sympathetic nerve activity resulting in decreased thermogenesis (42) and/or activation of the hypothalamic thermoregulatory center (43). Furthermore, neuronal deletion of GHSR increases energy expenditure, locomotor activity and thermogenesis (44). The physiological significance of the observed effect of AG on Temp needs further investigation. No studies have assessed the effects of ghrelin on RR. GHSR is expressed in brain centers that control respiration (i.e. medulla oblongata and pons) (45), and more relevant to our other findings,
respiration is controlled by the autonomic nervous system. We speculate that the decreased RR with AG at least partly results from decreased sympathetic outflow or increased parasympathetic tone.

We also examined hormonal mechanisms through which AG or DAG could affect BP and HR. Changes in catecholamine levels and the renin–angiotensin–aldosterone system in response to sympathetic signaling are key mediators of short-term cardiovascular homeostasis. Previous studies have shown increased levels of Epi, ACTH and cortisol with AG administration in humans (19, 36). However, we neither observe significant changes in plasma Epi with AG infusion nor did we see changes in NE or renin levels. DAG infusion did not affect any hormone levels in our study, which is consistent with previous findings that DAG did not alter ACTH, GH or prolactin levels in humans (46).

However, we did observe an increase in aldosterone levels with AG infusion, a finding that has multiple potential explanations. AG is known to stimulate the release of pituitary hormones including growth hormone, prolactin and ACTH (46). We have also reported previously that AG, but not DAG, stimulates cortisol secretion (11). Although ACTH levels were not measured during this study, the elevated cortisol levels support an increase in ACTH, which may have stimulated aldosterone release as well. Consistent with this hypothesis, although AG fails to stimulate aldosterone secretion from freshly isolated adenocortical cells (47), GOAT inhibition has been shown to decrease plasma ACTH, aldosterone and corticosterone levels in vivo in rats (48).

Alternatively, decreased preload resulting from the AG infusion may have decreased levels of the natriuretic peptides, atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP). The natriuretic peptides are important inhibitors of aldosterone secretion (49); thus, lower levels may have resulted in increased aldosterone. Because the natriuretic peptides can also inhibit catecholamine release as well as renin (49), we might have expected to see similar rises in these hormones. However, a study of salt-induced hypertension in rats also found no effect of chronic ghrelin administration on renin or urine catecholamine levels, despite improved BP in the ghrelin-treated group (50). Although aldosterone levels were not measured in that study, ghrelin treatment did significantly decrease ANP mRNA expression in the myocardium of the high salt group (50). Additional work is needed to define the effects of AG-related BP changes on natriuretic peptide and aldosterone levels.

With a clinical trial reporting beneficial effects of DAG on glucose metabolism distinct from the actions of AG (13), we sought to explore whether DAG could also exert GHSR-independent effects on BP. To our knowledge, prior investigations assessing the CV effects of DAG have been limited to animal models and in vitro settings. DAG was comparable to AG in reversing endothelin-1-induced vasoconstriction in isolated human coronary arteries (15) and decreasing MAP and HR when administered centrally to the NTS in rats (51). More recently, lower levels of DAG have been associated with an increased risk of CV events (26) in older hypertensive humans as well as increased BP in men with hypertension (25). We show that in humans, peripheral DAG infusion did not alter systemic BP, RR or Temp acutely or counteract the effects of AG. Therefore, the observed ghrelin effects on the CV system are likely to be GHSR dependent. Interestingly, DAG infusion alone did lead to a modest decrease in HR compared to saline control with no significant changes in BP. This isolated bradycardic effect of DAG has not been seen previously in humans and could be mediated by DAG-specific receptors on cardiomyocytes, which have been described in mice (52). Future studies are needed to confirm this finding.

There are limitations of our study. First, BP, HR, RR and Temp measurements were obtained as part of a study that involved an insulin-modified frequently sampled IVGTT. The IV glucose bolus (11.4 g/m²) and/or insulin infusion (0.025 units/kg) could have affected our CV parameters. However, as the IVGTT occurred on all study days, any confounding effects should have been equivalent on all days. Secondly, the hormonal measures (i.e. Epi, NE, renin and aldosterone) were only obtained in 10 of the 17 participants. Therefore, as mentioned previously, we cannot exclude a type II error in our negative findings on catecholamine or renin secretion.

Overall, our study confirms past findings that AG infusion decreases systemic BP in healthy humans and yields additional findings of decreases in HR, RR, and Temp. We believe the most likely explanation for these CV effects is through CNS actions of AG to depress sympathetic tone and/or stimulate parasympathetic activity. Furthermore, we report the novel finding that continuous DAG infusion in humans does not significantly change BP nor does it counteract the CV effects of AG. However, DAG did mildly lower HR, which suggests a GHSR-independent mechanism and may have clinical implications, as GOAT inhibitors and DAG mimetics are being actively pursued as therapeutic options for type 2 diabetes and obesity. These ongoing trials highlight the importance of understanding the effect of ghrelin on the CV system.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
Funding for this research is provided by NIH/NIDDK (R03DK89090 and R01DK097550 to J T and R0157990 to D D) and supported in part by USPHS grant U11 RR26314 from the National Center for Research Resources, NIH. L P was supported by NIH training grants (5T32AI007217-33 and 4T32HD043029-14).

Author contribution statement
C Z wrote the manuscript and contributed to the discussion. M B contributed to the assay performance, the discussion and editing of the manuscript. M A analyzed the data. L P contributed to the discussion and reviewed/edited the manuscript. D D contributed to the discussion and reviewed/edited the manuscript. M T contributed to the discussion and reviewed the manuscript. J T designed the study, collected the data and reviewed/editing the manuscript.

References
22 Lin Y, Matsumura K, Fukushima T, Nagai S, Fujita K & Iida M. Ghrelin acts at the nucleus of the solitary tract to decrease
arterial pressure in rats. Hypertension 2004 43 977–982. (doi:10.1161/01.HYP.0000122803.91559.55)


38 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. Journal of Pharmacology and Experimental Therapeutics 2002 302 822–827. (doi:10.1124/jpet.102.034827)


Received 16 September 2016
Revised version received 2 November 2016
Accepted 8 November 2016