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Angiotensin II-induced Reduction in Body Mass Is Ang II Receptor-Mediated in Association with Elevated Corticosterone

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Abstract

The mechanisms by which elevated glucocorticoids contribute to decreased body mass via angiotensin II (Ang II) infusion are not completely described. This study addressed the hypothesis that chronic Ang II infusion suppresses hepatic growth hormone receptor (GHR) and IGF1 expressions via an Ang II receptor (AT1)-mediated pathway associated with elevated glucocorticoids. Sprague-Dawley rats were assigned to three groups: 1) Control, 2) Ang II-infused (80 ng/min $\times$ 28d) and 3) Ang II + angiotensin receptor blocker (ARB; 10 mg losartan/kg/d $\times$ 21d). After 28 d, Ang II decreased body mass by 14% (407 ± 8 vs 350 ± 17 g) and hepatic AT1a, GHR, and IGF1 mRNA expressions by 45%, 44%, and 44%, respectively. ARB treatment completely prevented the loss in body mass (409 ± 9 g) and AT1a and GHR expressions and partially recovered the loss of hepatic IGF1. Ang II increased plasma corticosterone (B) 3-fold (173 ± 28 vs. 555 ± 42 ng/mL) and ARB treatment prevented the response (150 ± 47 ng/mL). Food consumption did not change suggesting that the decrease in body mass resulted from the catabolic actions of the Ang II-induced increase in systemic B and not from reduced caloric intake. The prevention by ARB treatment of the Ang II-induced decrease in body mass and downregulation of AT1a, GHR and IGF1 coinciding with suppression of plasma B suggests that the Ang II-induced decrease in body mass is AT1 receptor mediated in conjunction with elevated B. These data suggest that alleviating the Ang II-induced cachexia requires targeting AT1 and suppressing glucocorticoid secretion.

Keywords

angiotensin receptor; cachexia; glucocorticoids; growth hormone; IGF1; proteolysis

Introduction

Infusion of angiotensin II (Ang II) induces a loss of lean tissue or skeletal muscle (cachexia) that is attributed to a decrease in systemic and muscle insulin-like growth factor 1 (IGF1) [1–13]. The Ang II-induced reduction in body mass (BM) can be alleviated by treatment

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with an angiotensin receptor blocker (ARB) suggesting that the mechanism of mass loss is angiotensin receptor mediated (AT1a) [7,8]. In addition, the ubiquitin-proteasome pathway of proteolysis, which is stimulated by elevated glucocorticoids, appears to be the major catabolic mechanism by which Ang II induces increased skeletal muscle atrophy, and thus reduced body mass [3,11,13–20]. Furthermore, acute dexamethasone (glucocorticoid) treatment inhibits growth hormone receptor (GHR) and IGF1 mRNA levels in cultured hepatocytes [21] and in rats [22], despite being reported to increase hepatic GHR mRNA levels in rabbits [23]. In general, glucocorticoids predominantly blunt pituitary GH responsiveness and antagonize GH actions, especially the hepatic production of IGF1 [21,22]. However, the effects of chronic Ang II infusion on hepatic GH-IGF1 axis and systemic glucocorticoids in relation to reduced body mass have not been fully examined.

Cachexia is a common consequence of many disease states such as congestive heart failure (CHF), which is associated with increased activation of the renin-angiotensin system (RAS) [4,11,13,24]. Thus, the infusion of Ang II has been developed as a model to address the biological actions of Ang II during conditions simulating CHF-associated cachexia [7–9,13]. Because a majority of the data has focused on the effects of Ang II on IGF1, primarily in skeletal and cardiac muscle [5–7,12,13,25], data on the hepatic effects of Ang II on GH-IGF1 axis are scarce. Under normal conditions, the pituitary secretion of GH stimulates the release of IGF1 from the liver via activation of GHR [26,27]. The circulating concentrations of IGF1 are predominantly derived from hepatic production and secretion [28]. Therefore, the suppression of IGF1 by Ang II maybe associated with a concomitant decrease in GHR; however this remains to be elucidated. In the 2-kidney, 1-clip (2K, 1C) hypertensive rat, left ventricular GHR and IGF1 mRNA expressions were upregulated in response to the pressure-overload associated with this form of renal hypertension [25]. However, this is not surprising as it is well-established that Ang II infusion is associated with upregulation of cardiac IGF1, which likely contributes to the cardiac hypertrophy observed in this model [5,11–13,25]. In addition, because Ang II infusion did not alter hepatic AT1a expression [29], quantification of hepatic AT1a mRNA expression will help elucidate if reduced BM is angiotensin receptor-mediated since treatment with ARB was shown to alleviate the decrease in BM.

To address the hypothesis that chronic infusion of Ang II suppresses hepatic GHR and IGF1 expressions via an AT1a-mediated pathway that is associated with elevated glucocorticoids, we quantified the effects of chronic (28 d), low-dose (80 ng/min) Ang II infusion on 1) hepatic mRNA expression of GH receptor (GHR), IGF1 and AT1a, and 2) urinary and plasma corticosterone (B) concentrations.

Methods

All procedures were approved by the Institutional Animal Care and Use Committees of Tulane University Health Sciences Center and the University of California, Merced.

Animals and study procedures

The present paper consisted of two independent study cohorts (Study 1 and Study 2) of male, Sprague-Dawley rats (200–225 g; Charles River, Wilmington, MA). Study 1 was conducted to insure that chronic Ang II infusion induced a sustainable increase in corticosterone as determined by urinary B excretion and to assess the effects of chronic Ang II infusion hepatic GH-IGF1 axis. Given the results from Study 1, Study 2 was conducted to assess the contribution of AT1-mediated processes by including a group of animals simultaneously treated with an angiotensin receptor blocker (ARB).

In Study 1, rats were randomly assigned to either a Control (n = 10) or an Ang II-infused group (80 ng/min $\times$ 28 d; n = 10). In Study 2, rats were randomly assigned to three groups: 1) Control (n = 8), 2) Ang II-infused (80 ng/min $\times$ 28 d; n = 7), or 3) Ang II-infused + ARB (10 mg losartan/kg/d in the diet $\times$ 21 d as of day 7; n = 7). In both studies, control animals were sham operated and Ang II-infused animals were implanted with osmotic mini-pumps (Durect Corp., Cupertino, CA; model 2004) infusing Ang II (Phoenix Pharmaceuticals, Belmont, CA). The present study incorporated a relatively low dose of Ang II for a longer period than that commonly used to gain further insight of the dose and temporal effects of Ang II on body mass [5–8,10,13,25]. Following these procedures animals were placed in metabolic cages and allowed to acclimate to the cages for 3 days prior to the initiation of data collection. All animals were maintained on the same diet (Purina rodent chow #5002) with the exception of the Ang II + ARB group, which was switched to their ARB-treated diet after the first week of Ang II infusion.

In Study 1, body mass was recorded daily, and food and water consumption and urine output were recorded daily for the first 16 d and then intermittently on days 19, 22, 25, and 28. In study 2, body mass was recorded daily, and food and water consumption and urine output were measured weekly. Urinary corticosterone concentrations were only measured from aliquots collected on the last day in Study 2.

Dissections

Following the final measurements on day 28, animals were decapitated and trunk blood was collected into chilled vials containing 5 mM EDTA plus protease inhibitor cocktail (pH = 7.4, 12.5 mM 1-10-phenanthroline, 22 μ M pepstatin, 10 μ M PMSF, 20 μ M enalaprilat). In Study 1, the heart, liver, and retroperitoneal and epididymal fat were removed, blotted dry, and weighed. Immediately after weighing the liver a piece (approximately 200 mg) was cut and placed in a vial of RNA Later (Ambion, Austin, TX) for later quantification of AT1a, GHr and IGF1 mRNA expressions by qRT-PCR (described later). In Study 2, an additional measure of the gastrocnemius muscle was taken to evaluate the effects of chronic Ang II infusion on skeletal muscle (lean tissue) catabolism.

Hormone Analyses

Urine samples were extracted by dichloromethane for measurement of B (MP Biomedicals, Solon, OH) as previously described [30]. Urinary excretion of B ($U_B V$) was calculated as the product of concentration and urine volume. Plasma was used to measure B (MP Biomedicals), GH (Linco), ghrelin (Phoenix Pharmaceuticals), and testosterone (T; DPC) in Study 1. Because plasma ghrelin and T were not significantly altered in Study 1, we did not repeat these measures in Study 2, and only proceeded with B and GH measures. Furthermore, the lack of a change in food consumption in both studies did not justify any further measures of ghrelin. Hormones were measured by radioimmunoassay, and all samples were run in a single assay with intra-assay % coefficient of variation of between 5 and 11 % using internal controls to calculate %CV.

qRT-PCR

Real-time qRT-PCR was performed as previously described [31]. In brief, total RNA was extracted from the piece of liver using a commercially available kit (Qiagen, Valencia, CA), and treated with DNase I (Invitrogen, Carlsbad, CA) to remove contaminated genomic DNA. Quantitative real-time RT-PCR was conducted using an Mx3000P (Stratagene, Santa Clara, CA) with MxPro QPCR software (Stratagene), and Brilliant 1-Step QRT-PCR master mix kit as reagent. Dual label was 5': 5-HEX, 3': BHQ-2 for control gene and 5': 6-FAM, 3': BHQ-1 for target genes. Program conditions were Step 1: 50°C, 30 min, 1 cycle; Step 2: 95°C, 10 min, 1 cycle; and Step 3: 95°C, 15 sec followed by 60°C, 1 min, 40 cycles. The

base pair sequences of the primers and probes used to quantify AT1a, GHr and IGF-1 mRNA expressions are listed in Table 1. The primers recognized total GHr and IGF1 mRNA and did not distinguish among any variants of these proteins. The quantity of the mRNA expressions was normalized by GAPDH mRNA expression.

Statistics

Because the Control and Ang II groups were treated similarly in both studies, we first compared means by analysis of variance for both groups between the two studies to determine if an inter-study effect was present. When it was determined there was not an inter-study effect, data from Control and Ang II groups were combined and these means compared to that for the Ang II + ARB group. The means for body mass were not combined, and presented as separate data sets for the two studies to demonstrate and emphasize the consistent effect of the Ang II-infusion on body mass. Means ($\pm$ SE) of daily measurements were compared by analysis of variance adjusted for repeated measures over time. If significant ($p < 0.05$) group $\times$ time interactions were detected, one-way analysis of variance was performed to determine differences on specific days. Means for plasma and urinary, body and organ mass, and relative mRNA expression measurements were compared by one-way analysis of variance. Correlations were determined by simple regressions. For all cases, if significance ($p < 0.05$) was detected, a Fisher's Protected Least Significant Difference test was applied post-hoc. Statistics were performed using Statview software (SAS, Cary, NC).

Results

Body Mass

In Study 1, at the beginning of data collection on day 4, mean body mass was not different ($p > 0.10$) between the two groups (273 ± 3 vs 265 ± 4 g). Ang II induced a significant group $\times$ time interaction ($p < 0.01$) with mean BM deviating significantly ($p < 0.05$) from Control on day 14 and remaining reduced ($p < 0.01$) for the rest of the study (Figure 1A). Infusion of Ang II decreased mean BM by 10% (406 ± 10 vs 365 ± 13 g) by day 28 (Figure 1A). In Study 2, at the beginning of data collection on day 4, mean body mass was not different ($p > 0.10$) among the three groups (Control: 272 ± 8 , Ang II: 263 ± 3 and Ang II + ARB: 255 ± 9 g). Ang II induced a significant group $\times$ time interaction ($p < 0.01$) with mean BM deviating significantly ($p < 0.05$) from Control on day 8 and Ang II + ARB on day 15 remaining reduced ($p < 0.01$) for the remainder of the study (Figure 1B). A significant group $\times$ time interaction between Control and Ang II + ARB groups was not detected ($p > 0.10$) suggesting that ARB treatment alleviated the Ang II-induced decrease in body mass (Figure 1B). Infusion of Ang II decreased mean BM by 14% (407 ± 8 vs 350 ± 17 g) compared to Control and by 12% (409 ± 9 g) compared to Ang II + ARB by day 28 (Figure 1B).

Food & Water Consumption

Initially, mean absolute and relative food consumption in the Ang II + ARB group was lower ($p < 0.05$) than Control, but this decrease was not associated with reduced body mass and was not observed after the first week through to the end of the study (Table 2). No time or group $\times$ time interactions ($p > 0.10$) in either mean absolute or relative food consumption was detected among the three groups; therefore only the initial (day 4) and ultimate (day 28) values for these parameters are presented in Table 2. Mean absolute and relative water consumption on day 4 was not different ($p > 0.10$) among the three groups (Table 2). Ang II induced a significant group $\times$ time interaction ($p < 0.001$) with increases in mean absolute water consumption observed on days 5 and 9, and from day 11 through the remainder of the study. Ang II infusion increased mean absolute and relative water consumption 68% and 87%, respectively, by day 28 (Table 2). Treatment with ARB completely reversed the Ang

II-induced polydipsia after the first week of treatment and mean absolute and relative water consumption remained at Control levels for the remainder of the study (Table 2).

Urine Output

Similar to water consumption, mean urine output was not different on day 4 ($p > 0.05$) among the three groups. A significant group $\times$ time interaction was detected ($p < 0.001$) within the first week of Ang II infusion between the Control and Ang II groups with Ang II inducing an increase in mean urine output that remained elevated for the remainder of the study (Table 2). Reduction of the polyuria by ARB treatment was not observed until the 2nd week of treatment and these levels remained lower ($p < 0.05$) than Ang II for the duration of the study (Table 2). The changes in urine output paralleled those in water consumption with water consumption significantly and positively correlated with urine output (urine output = water consumption $\times$ 0.89 – 16; $R = 0.985$; $p < 0.0001$).

Organ Masses

Mean absolute liver and heart mass was not different ($p > 0.10$) among the three groups. Ang II infusion decreased ($p < 0.05$) mean absolute gastrocnemius, retroperitoneal fat, and epididymal fat masses by 28%, 49% and 30%, respectively, and ARB treatment prevented these mass losses.

Ang II increased ($p < 0.05$) mean relative liver (Figure 2, insert) and heart masses by 7% and 26%, respectively (Figure 2). The decrease in mean relative liver mass associated with ARB treatment did not quite reach significance ($p = 0.065$) (Figure 2, insert), but ARB treatment completely alleviated the cardiac hypertrophy (Figure 2). Ang II infusion decreased ($p < 0.01$) mean relative gastrocnemius mass by 16% and ARB treatment prevented the decrease and returned mean values to control levels (Figure 2). Ang II infusion decreased mean relative retroperitoneal fat by 47% ($p < 0.01$) and epididymal fat ($p < 0.05$) by 24% (Figure 2). Treatment with ARB prevented ($p < 0.05$) the Ang II effects on retroperitoneal fat, but not on epididymal fat (Figure 2).

Relative qRT-PCR Expressions

Ang II infusion decreased ($p < 0.001$) the mean relative mRNA expressions of AT1a, GHr, and IGF1 by 45%, 44%, and 44%, respectively (Figure 3). Treatment with ARB completely prevented the effects of Ang II on AT1a and GHr mean relative mRNA expressions. ARB treatment only partially abated the Ang II-induced downregulation of mean relative IGF1 expression by increasing levels by 30% ($p < 0.05$) above Ang II, but levels remained 27% lower ($p < 0.01$) than Control (Figure 3).

Hormones

Ang II increased ($p < 0.001$) plasma B 3.2-fold on day 28 and ARB treatment completely prevented ($p < 0.001$) this increase (Figure 4A). Similar to the Ang II effects on plasma B, Ang II increased ($p < 0.001$) urinary B excretion 2.2-fold on day 28 that was absent ($p < 0.001$) in the ARB treated group (Figure 4B, insert). A significant group $\times$ time interaction in U_BV was detected with significant Ang II-induced increases observed on day 8 ($p < 0.05$) and from day 15 ($p < 0.01$) through the remainder of the study (Figure 4B). When the percent difference in mean U_BV and mean BM between Control and Ang II groups for each day was compared by simple regression, a significant and positive correlation was observed (% change in BM = % change in $U_BV \times 0.03 + 2$; $R = 0.744$; $p < 0.001$) (Figure 5).

Ang II suppressed ($p < 0.01$) plasma GH (24.5 ± 5.9 vs. 3.0 ± 1.1 ng/mL), but the increase associated with ARB treatment (10.4 ± 3.8 ng/mL) was not significant. No significant

differences between Control and Ang II groups were detected in plasma ghrelin (61 ± 4 vs. 77 ± 7 pM) and T (9.0 ± 2.0 vs. 10.8 ± 2.9 nM) levels.

Discussion

Decreased body mass is a well-established consequence of elevated Ang II that serves as a model for evaluating the cachexia associated with many disease states such as CHF [2,4–8,10,11,13,15,25]. While an increase in cardiac IGF1 likely promotes the cardiac hypertrophy commonly associated with elevated Ang II, suppressed skeletal IGF1 and increased ubiquitin-proteasome pathway contribute to the Ang II-induced skeletal muscle atrophy [3,6,11,13]. Treatment with an ARB alleviates the Ang II-induced decrease in body mass suggesting that the effects of Ang II are angiotensin receptor-mediated [7,8]; however, mediation of the Ang II effects on body mass via AT1 has not been fully examined. The present study revealed that the Ang II effects on hepatic GH-IGF1 axis and skeletal muscle atrophy are likely attributed to increased systemic B. Secondly, the ability of ARB treatment to reverse the Ang II-induced decreases in hepatic GHr and IGF1 suggests that the suppression of the hepatic GH-IGF1 axis is AT1a-mediated. The low and chronic dosage protocol used in the present study was not associated with caloric restriction and thus did not necessitate paired-feeding as in other studies in which higher and more acute dosing protocols were used [2,5–8,13].

The effects of Ang II on glucocorticoid secretion are not consistent and data on the effects of chronic Ang II infusion on glucocorticoids are scarce. In men, acute (120 min) Ang II infusion either reduced or had no change on plasma cortisol (F) and B [32]. In rats, acute (30–40 min) Ang II infusion produced selective stimulation of aldosterone without a parallel increase in B secretion [33] and chronic (21 d) infusion did not increase plasma B [34]. However, acute (90 min) infusion of Ang II increased plasma B in a dose-dependent manner in spontaneously hypertensive rats [35]. Brink et al.[6] reported that B excretion was high in *some* Ang II-infused rats, but that the responses were quite variable, but unfortunately, no values were reported, limiting the interpretation of this data. The present study clearly demonstrates that Ang II induces an increase in U_BV that is maintained elevated over the last 2 weeks of the study and is associated with a 3-fold increase in plasma B. The ability of ARB treatment to prevent the Ang II-induced increase in B indicates that its secretion is AT1 mediated.

Furthermore, the contribution of glucocorticoids to the ubiquitin-proteasome proteolytic pathway in the Ang II-infused model of cachexia is not well established. The positive and significant correlation between the percent change in mean U_BV and mean BM suggests that the decrease in body mass is associated with an increase in systemic B. Interestingly, the day that mean BM began to deviate from control levels coincided with the day in which mean U_BV began to increase. The Ang II-induced increase in B likely contributed to the decrease in BM by increasing protein degradation via the ubiquitin-proteasome pathway since it has been well established that elevated glucocorticoids promote proteolysis via this mechanism [3,14,16–18,20,36]. The decrease in the mass of the gastrocnemius induced by Ang II suggests that skeletal muscle atrophy is associated with elevated systemic B. The ability of ARB treatment to prevent the lost skeletal muscle mass suggests that the corticosterone-associated atrophy is AT1 mediated. The transfer of IGF1 by electroporation prevents skeletal muscle atrophy in glucocorticoid-treated rats [3] suggesting that inhibition of the Ang II-induced increase in circulating B may be a critical target of future therapies to help alleviate the cachexia observed in many disease states associated with elevated Ang II, and to recover the suppressed GH-IGF1 axis.

Contributing to the decrease in body mass was the suppression of hepatic GHr and IGF1 mRNA by Ang II. Ang II induces differential responses to IGF1 expression in tissues. While Ang II increases cardiac IGF1, which likely promotes cardiac hypertrophy [5,25], it also suppresses hepatic IGF1 expression, which results in reduced circulating levels [6,7]. Whether the suppression of hepatic IGF1 by Ang II is receptor mediated or the indirect effect of another factor such as glucocorticoids remains to be resolved as indicated by Brink et al. in their recent review [4]. The ability of ARB treatment to completely recover GHr and partially recover IGF1 mRNA expressions from Ang II-induced downregulation suggests that regulation of hepatic GHr-IGF1 is AT1a-mediated. The parallel changes in mRNA expressions and plasma B following treatment with ARB suggests that B also contributes to the regulation of hepatic GHr-IGF1. This is further corroborated by the fact that dexamethasone reduces hepatic IGF1 mRNA [21,22]. Because GH stimulates hepatic IGF1 secretion via activation of hepatic GHr, the downregulation of hepatic GHr also likely contributed to the decrease in hepatic IGF1 mRNA.

The lack of a change in food consumption among the groups indicates that the decrease in BM was not due to caloric restriction but rather by the direct and indirect effects of elevated Ang II and B. Moreover, few data exist on the contributions of altered body fat distribution to the decrease in body mass during Ang II-infusion [10]. The present study demonstrated that Ang II infusion significantly decreased epididymal and retroperitoneal fat depots; however these decreases contributed to less than 15% of the reduction in BM suggesting that lean tissue loss is the major and primary component of the mass loss. The decrease in gastrocnemius mass indicates that skeletal muscle loss is significant and likely accounts for the body mass loss not attributed to lost fat mass. While the loss of total body water may also contribute to the decrease in BM, the correlation between water consumption and urine output suggests that output paralleled input resulting in virtually no net loss of water.

The effects of Ang II infusion on circulating ghrelin have not been examined. The stimulation of food intake and GH secretion by the gastric peptide, ghrelin, is well recognized [37,38]. Therefore, the lack of a change in food intake coincides with the lack of a change in plasma ghrelin in the present study. In CHF patients experiencing cachexia and exhibiting elevated plasma Ang II levels, plasma ghrelin was higher than in CHF patients without cachexia, which led the authors to suggest that this increase in ghrelin represented a compensatory mechanism to stimulate caloric intake to counter the catabolic imbalance [39]. While plasma ghrelin was not increased following chronic Ang II infusion, the increase in plasma B may have suppressed the peripheral signal to significantly increase plasma ghrelin. Endogenously and exogenously elevated glucocorticoids have been reported to decrease plasma ghrelin in humans [40]. Thus, in the present study, it would appear that ghrelin secreting gastric cells were receiving competing signals of stimulation and suppression, resulting in the lack of a significant increase in plasma ghrelin, and ultimately, food intake.

In conclusion, the present study suggests that the Ang II-induced reduction in body mass is attributed to increased circulating B. While the effects of acute and chronic Ang II on B secretion are inconsistent, the present study demonstrates that chronic Ang II infusion increases plasma B via AT1. In addition, the Ang II-induced suppression of hepatic IGF1 mRNA is likely attributed to increased systemic B and downregulation of hepatic GHr mRNA. The ability of ARB treatment to restore (either completely or partially) GHr and IGF1 mRNA expressions, and body mass while preventing the Ang II-induced increase in plasma B suggests that the cachexia is AT1-mediated and attributed, at least in part, to elevated B. The lack of a change in food intake suggests that caloric restriction does not contribute to reduced body mass in this low dose, chronic Ang II-infusion protocol. Ang II infusion reduced skeletal muscle and, retroperitoneal and epididymal fat depots; however the decreases in these primary fat depots contributed to less than 15% of the decrease in

body mass suggesting that increased lean tissue atrophy is the primary contributing factor to the loss of body mass. Because it is well established that elevated glucocorticoids promote proteolysis by the ubiquitin-proteasome pathway, the increase in plasma B and U_BV suggests that this pathway was primarily responsible for the increased loss of lean tissue. The present study demonstrates that simultaneously targeting the angiotensin receptor (primarily AT1) and suppressing glucocorticoid secretion may be the best course of action to alleviate the cachexia associated with pathophysiological disorders in which angiotensin II is inappropriately elevated.

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Figure 1. Mean ($\pm$ SE) body mass of **A)** Control and Angiotensin II (Ang II)-infused rats ($n = 10$ /group) in Study 1, and **B)** Control ($n = 8$), Ang II ($n = 7$) and Ang II + angiotensin receptor blocker (ARB; $n = 7$) groups in Study 2. Ang II was infused (80 ng/min) for 28 d and ARB treatment (10 ng/kg/d in the diet) was initiated after the first week of Ang II infusion. * denotes significant ($p < 0.05$) difference from Control. Control and Ang II + ARB groups were not different.

Figure 2.

Mean ($\pm$ SE) relative organ masses from Control (n = 18), Angiotensin II (Ang II; n = 17), and Ang II + angiotensin receptor blocker (ARB; n = 7) groups. Ang II was infused (80 ng/min) for 28 d and ARB treatment (10 ng/kg/d in the diet) was initiated after the first week of Ang II infusion. * denotes significant (p < 0.05) difference from Control and # denotes significant (p < 0.05) difference from Angiotensin II. RP fat = retroperitoneal fat. Epi fat = epididymal fat pads.

Figure 3.

Mean ($\pm$ SE) relative units of mRNA expressions of hepatic angiotensin receptor type 1a (AT1a), growth hormone receptor (GHr), and insulin-like growth factor -1 (IGF1) from Control (n = 12), Angiotensin II (Ang II; n = 10), and Ang II + angiotensin receptor blocker (ARB; n = 6) groups. Ang II was infused (80 ng/min) for 28 d and ARB treatment (10 ng/kg/d in the diet) was initiated after the first week of Ang II infusion. * denotes significant ($p < 0.05$) difference from Control and # denotes significant ($p < 0.05$) difference from Angiotensin II.

Figure 4. Mean ($\pm$ SE) **A**) plasma corticosterone (**B**) from Control ($n = 17$), Angiotensin II (Ang II; $n = 14$), and Ang II + angiotensin receptor blocker (ARB; $n = 6$) groups and **B**) urinary B excretion from Control ($n = 10$) and Angiotensin II (Ang II; $n = 10$) groups from Study 1. **B**) *Insert:* Mean ($\pm$ SE) urinary B excretion from Control ($n = 7$), Angiotensin II (Ang II; $n = 6$), and Ang II + angiotensin receptor blocker (ARB; $n = 6$) groups on day 28 of Study 2. Ang II was infused (80 ng/min) for 28 d and ARB treatment (10 ng/kg/d in the diet) was initiated after the first week of Ang II infusion.. * denotes significant ($p < 0.05$) difference from Control and # denotes significant ($p < 0.05$) difference from Angiotensin II.

Figure 5.

Correlation between the percent change in urinary corticosterone excretion and the percent change in body mass between Control and Angiotensin II (Ang II)-infused rats (n=10/group) for each day during Study 1. Ang II was infused (80 ng/min) for 28 d. The regression was considered significant at $p < 0.05$.

Table 1

The base pair sequence of the primers (Forward and Reverse) and probes for the qRT-PCR quantification of hepatic angiotensin type 1a receptor (AT1a), growth hormone receptor (GHR), insulin-like growth factor-1 (IGF1), and GAPDH.

Sequence	
<u><i>AT1a</i></u>	
Forward	CTTGTTCCCTTTCCTTATCA
Reverse	CGTTTCTGGTTTGTTCTTT
Probe	CACCAGCTATACCCTTATTGGAAAGC
<u><i>GHR</i></u>	
Forward	AGCTGTTATGACCCTGACAT
Reverse	CAAGAGATCAGCTTCTGCTT
Probe	ATACCGATTTCACACCAGTGACAT
<u><i>IGF1</i></u>	
Forward	TCTCTTCTACCTGGCACTCT
Reverse	GTCCACACACGAACTGAAG
Probe	TGCTCACCTTTACCAGCTCGG
<u><i>GAPDH</i></u>	
Forward	CAGAACATCATCCCTGCATC
Reverse	CTGCTTCACCACCTTCTTGA
Probe	CCTGGAGAAACCTGCCAAGTATGATGA

Table 2

Mean ($\pm$ SE) metabolic parameters initially (day 4) and at the end of the study (day 28) from Control, Angiotensin II (Ang II), and Ang II + angiotensin receptor blocker (ARB) groups.

	Control	Angiotensin II	Ang II + ARB
<i>Absolute Food Consumption (g/d)</i>			
Day 4	27 $\pm$ 1	23 $\pm$ 2	22 $\pm$ 1 *
Day 28	26 $\pm$ 1	26 $\pm$ 2	27 $\pm$ 2
<i>Relative Food Consumption (g/100 g BM/d)</i>			
Day 4	7.8 $\pm$ 0.5	7.9 $\pm$ 0.6	4.1 $\pm$ 0.1 *,#
Day 28	6.3 $\pm$ 0.2	6.8 $\pm$ 0.3	6.6 $\pm$ 0.4
<i>Absolute Water Consumption (mL/d)</i>			
Day 4	35 $\pm$ 1	41 $\pm$ 3	37 $\pm$ 2
Day 28	40 $\pm$ 2	67 $\pm$ 4 *	39 $\pm$ 4 #
<i>Relative Water Consumption (mL/100g BM/d)</i>			
Day 4	8.5 $\pm$ 0.5	9.8 $\pm$ 0.8	9.0 $\pm$ 0.6
Day 28	9.8 $\pm$ 0.7	18.3 $\pm$ 3.4 *	9.6 $\pm$ 0.9 #
<i>Urine Output (mL/d)</i>			
Day 4	15 $\pm$ 2	20 $\pm$ 2	16 $\pm$ 2
Day 28	20 $\pm$ 2	43 $\pm$ 3 *	23 $\pm$ 3 #

* denotes significant ($p < 0.05$) difference from Control

denotes significant ($p < 0.05$) difference from Angiotensin II