Mechanisms contributing to angiotensin II regulation of body weight

LISA A. CASSIS, DANA E. MARSHALL, MICHAEL J. FETTINGER, BRADY ROSENBLUTH, AND ROBERT A. LODDER
Divisions of Pharmacology and Experimental Therapeutics and Medicinal Chemistry and Pharmaceutics, College of Pharmacy, University of Kentucky, Lexington, Kentucky 40536-0082

The renin-angiotensin system (RAS) is central to cardiovascular function and plays a key role in the regulation of body weight. In adipose tissue, the RAS has been shown to be involved in the regulation of fat mass, metabolism, and thermogenesis. The angiotensin II (ANG II) receptor agonist, losartan, has been shown to decrease body weight and improve metabolic parameters in obese rodent models. The mechanisms by which ANG II regulates body weight are not well understood, and further investigation is needed to elucidate the role of the RAS in adipose tissue metabolism.

Methods

The study was designed to investigate the effects of ANG II on body weight and energy expenditure in rats. Three different protocols were used: study 1, study 2, and study 3. In study 1, ANG II infusion (350 ng·kg⁻¹·min⁻¹) was initiated into rats for 14 days. In study 2, the effect of ANG II infusion on body weight, food and water intake, and blood pressure was examined. ANG II or saline (n = 5/group) was infused into rats for 14 days. In study 3, the effect of chronic low-dose (175 ng·kg⁻¹·min⁻¹) and high-dose (500 ng·kg⁻¹·min⁻¹) ANG II infusion on body weight, food and water intake, and blood pressure was examined. ANG II or saline (n = 5/group) was infused into rats for 14 days.

Results

In study 1, ANG II infusion resulted in a marked reduction (26%) in body weight. Food intake was not increased, but water intake was increased in ANG II-infused rats. Blood pressure gradually increased to significantly elevated levels by day 14. Thermal infrared imaging demonstrated an increase in abdominal surface temperature. Measurement of organ mass demonstrated site-specific reductions in white adipose tissue mass after ANG II infusion. In study 2, the dose-response relationship for ANG II infusion (200, 350, and 500 ng·kg⁻¹·min⁻¹) was determined. Body weight (decrease), blood pressure (increase), white adipose mass (decrease), plasma ANG II levels (increase), and plasma leptin levels (decrease) were altered in a dose-related manner after ANG II infusion. In study 3, the effect of ANG II infusion (350 ng·kg⁻¹·min⁻¹) was examined in rats treated with the vasodilator hydralazine. Hydralazine treatment normalized blood pressure in ANG II-infused rats. The effect of ANG II to decrease body weight was augmented in hydralazine-treated rats. These results demonstrate that low levels of ANG II infusion regulate body weight through mechanisms related to increased peripheral metabolism and independent of elevations in blood pressure.

Conclusion

The results of these studies suggest adipose tissue as a potential site for the local renin-angiotensin system, and ANG II regulation of body weight. These findings have important implications for the development of therapeutic interventions for the treatment of obesity and related metabolic disorders.
2, the dose-response relationship for ANG II infusion on body weight, blood pressure, and food and water intake was examined. Four groups of rats (n = 3/group) were examined for 7 days and infused with saline or ANG II at doses of 200, 350, and 500 ng·kg⁻¹·min⁻¹. In study 3, the effect of ANG II on body weight was examined in rats that were treated with the vasodilator hydralazine. Four groups (n = 4/group) of rats were examined for 7 days, saline infused with or without hydralazine, and ANG II infused with or without hydralazine. In all three studies, body weight and food and water intake were measured daily, and measurements of blood pressure were taken every 3–5 days.

ANGII infusion model. Male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) were used in all studies. Rats ranged in body weight from 350 to 450 g. All rats were housed two per cage in an approved animal facility for 1 wk before use under normal light-dark cycles and were given free access to food and water. During each experimental protocol, rats were housed individually for measurement of body weight and food and water intake on a daily basis at 10:00 AM. Baseline measurements of food intake, water intake, and blood pressure were performed on all rats in an individual study for a minimum of 3 days preceding each experimental protocol.

For ANG II infusion, rats were anesthetized with diethyl ether and shaved in the interscapular region; then osmotic minipumps (model 2002 for 14-day infusion, model 2001 for 7-day infusion: Alza, Palo Alto, CA) were implanted subcutaneously. Minipumps contained either ANG II (Sigma Chemical, St. Louis, MO; 175–500 ng·kg⁻¹·min⁻¹ infusion rate) or sterile saline and were primed according to the manufacturer’s instructions preceding implantation to assure immediate subcutaneous delivery of ANG II. The skin overlaying the minipump was closed with surgical staples, and rats were allowed to recover on warmed heating pads. Indirect systolic pressure by tail cuff plethysmography. In study 1, systolic pressure was measured in conscious restrained rats by use of a Narco system. In studies 2 and 3, systolic pressure was measured on ether-anesthetized rats using an inflatable tail cuff, a pressure and pulse transducer, and a recording polygraph. Alterations in blood pressure from anesthesia were controlled for across ANG II- and saline-infused rats. The systolic pressure from three separate measurements was averaged from each rat. Baseline systolic pressure was recorded for 3 days preceding implantation of osmotic minipumps. After implantation of ANG II-containing minipumps, blood pressure was measured every 3–5 days.

Hydralazine treatment. Hydralazine (15 mg/kg) was administered in the drinking water of individual rats in study 3. Hydralazine dosing was based on an average water consumption of 40–60 ml of water intake per day. The dose of hydralazine in the drinking water was adjusted daily on the basis of the preceding 24-h water intake and daily body weight measurements in individual rats.

Thermal infrared imaging. Thermal infrared (IR) imaging was used in study 1, as an index of peripheral energy expenditure. Thermal IR imaging was performed using a liquid nitrogen-cooled InSb focal plane array camera (temperature precision = 0.03°C; 3,000–5,000 nm; Cincinnati Electronics, Mason, OH) with sound annotation capability. No external light source was used in comparing heat radiation in the different rats, so the intensity of features in each rat image corresponded to the level of blackbody emission from the skin and fur. Temperature calibration was accomplished using a blackbody source closely coupled to a mercury thermometer. The IR images were collected as 1-s segments of real-time video and saved on computer disk. The IR video camera had a frame collection rate of 51.44 frames/s, making sample target immobilization unnecessary. Thermal IR heat radiation was determined in joules per second (W) using standard software based on Stefan’s Law.

Measurement of plasma ANG II. Trunk blood was collected in heparinized vacuum test tubes containing the following buffer: 0.15 mM Hepes, 1 mM NaCl, 0.1 mM EDTA, 0.2% bovine serum albumin (BSA), pH 7.4. The inhibitors in this buffer were added to eliminate breakdown of angiotensin peptides as well as further production of peptides during sample handling (4). Plasma was obtained by centrifugation (3,000 g) of blood at 4°C for 30 min. Plasma samples were partially purified using Sep-Pak C18 column chromatography (Waters, Milford, MA), with the columns preequilibrated with 4 ml of methanol, 4 ml of water, and 10 ml of buffer. Angiotensin peptides were eluted from the columns with 2 ml of methanol-water-trifluoroacetic acid (70:29:1). The eluate was evaporated overnight using a speed-vac (Savant). Plasma ANG II was measured in preextracted samples, which were reconstituted in 100 µl of ANG II RIA buffer (0.1 M K2HPO4, 3.0 mM EDTA, 0.15 M NaCl, 0.05% BSA, pH 7.2), sonicated for 5 min, and stored at −20°C. Angiotensin content in each sample was measured by ANG II RIA using a polyclonal ANG II antibody (kindly supplied by Dr. A. Freedlender, University of Virginia) exhibiting minimal cross-reactivity to ANG I (2%) and angiotensins 5–8 (4%) but 100% cross-reactivity to ANG III, angiotensin 3–8, and angiotensin 4–8. The sensitivity of the RIA was 2 pg/ml.

Measurement of plasma leptin. Blood was obtained as described above, and an aliquot (500 µl) of plasma was removed for measurement of plasma leptin levels by use of a commercial RIA kit (Linco Research, St. Louis, MO) with a rat leptin antibody. The sensitivity of the kit for rat leptin was 0.5 ng/ml and required 100 µl of rat plasma for assay.

Statistical analysis. For all studies, data are means ± SE. In study 1, data (blood pressure, body weight, food and water intake) were analyzed using a two-way ANOVA, with ANG II as a between-group factor and time of infusion as a within-group repeated measure. In study 2, data were analyzed using a two-way ANOVA, with ANG II dose as a between-group factor and time as a within-group repeated measure. In study 3, data were analyzed using a three-way ANOVA, with ANG II dose and hydralazine treatment as between-group factors and time as a within-group repeated measure. Post hoc analysis was performed using Duncan’s multiple range test.

RESULTS

Study 1. The purpose of this study was to determine the effect of chronic low-dose ANG II infusion on the regulation of body weight. In study 1, ANG II was administered at an infusion dose of 175 ng·kg⁻¹·min⁻¹ to rats for a period of 14 days. Plasma ANG II levels after 14 days of infusion were not significantly different between ANG II- and saline-infused rats (saline: 37.3 ± 1.5; ANG II: 34.3 ± 8.2 pg/ml). Measurements of systolic blood pressure demonstrated a significant between-group effect of ANG II [F(1,7) = 57.7, P < 0.001] and a significant interaction between ANG II and time of infusion [F(5,35) = 2.7, P < 0.05]. Systolic blood pressure was significantly increased in ANG II-infused rats over baseline (day 0) levels by day 1 of ANG II infusion (Fig. 1). Moreover, systolic pressure in ANG II-infused rats was increased compared with saline controls at day 1. Initial increases in blood pressure
(days 1 and 3) in ANG II-infused rats were followed by a return to levels not significantly different from saline-infused controls on days 4 and 7. At 14 days, systolic pressure was significantly increased in ANG II-infused rats over saline-infused controls and from baseline measurements in the ANG II group before pump implantation (day 0). Values are means ± SE of 5/group. *Significantly different (P < 0.05) from sham (saline-infused) controls; †significantly different (P < 0.05) from day 0 within each group.

Daily body weight measurements revealed a significant interaction between ANG II and time of infusion [F(15,105) = 3.1, P < 0.05]. The body weight of ANG II-infused rats was significantly different from saline-infused controls from day 8 of infusion through the remainder of the experimental protocol (Fig. 2A). Saline-infused rats gained 27 g in body weight over the 14-day experimental protocol; in contrast, ANG II-infused rats did not gain weight over the 14-day protocol. Thus the primary effect of low-dose ANG II infusion was to eliminate weight gain. The time course for the effect of ANG II on body weight (Fig. 2A) and mean arterial pressure (Fig. 1) illustrates that these two variables did not change in parallel.

The effect of ANG II infusion on body weight was not the result of reductions in food intake (Fig. 2B). After an initial transient drop in food intake at 1–2 days after minipump implantation, ANG II infusion did not result in significant alterations in food intake. In contrast, infusion of ANG II significantly increased water intake (Fig. 2C). Increases in water intake were significant by 3 days of ANG II infusion and were maintained throughout the ANG II infusion protocol. Moreover, increases in water intake occurred before ANG II-induced reductions in body weight.

Thermal IR imaging demonstrated an increase in regional surface temperature in the tail and abdomen/thorax area of ANG II-infused rats compared with saline controls (Fig. 3A). An IR image of an ANG II-infused rat is illustrated in Fig. 3B. The surface temperature in the abdomen of the ANG II-infused rat was of higher intensity than that of the saline-infused control rat (Fig. 3A).
Maintained their relative mass after ANG II infusion. The other organs examined were the epididymal white fat (RPF), retroperitoneal white fat (RPF), and the diaphragm (skeletal muscle). The other organs examined were the epididymal white fat (RPF), retroperitoneal white fat (RPF), and the diaphragm (skeletal muscle). The other organs examined were the epididymal white fat (RPF), retroperitoneal white fat (RPF), and the diaphragm (skeletal muscle). The other organs examined were the epididymal white fat (RPF), retroperitoneal white fat (RPF), and the diaphragm (skeletal muscle). The other organs examined were the epididymal white fat (RPF), retroperitoneal white fat (RPF), and the diaphragm (skeletal muscle). The other organs examined were the epididymal white fat (RPF), retroperitoneal white fat (RPF), and the diaphragm (skeletal muscle). The other organs examined were the epididymal white fat (RPF), retroperitoneal white fat (RPF), and the diaphragm (skeletal muscle).

Fig. 3. Thermal infrared (IR) imaging demonstrates an increase in tail and abdomen surface temperature. In study 1, rats were infused with saline or ANG II (175 ng·kg$^{-1}$·min$^{-1}$) for 14 days. Thermal IR imaging was performed on day 14 using an InSb focal plane array camera. Temperature calibration was accomplished using a blackbody source closely coupled to a mercury thermometer. Surface temperature was increased in the tail and abdomen from ANG II-infused rats compared with saline controls (A). *Significantly different (P < 0.05) from saline control. B: image of an ANG II-infused rat. Note visible detection of temperature in abdomen and tail.

A variety of organs were removed from ANG II-infused and saline-infused rats at the end of the experimental protocol, and organ weight-to-body weight ratios were constructed to determine sites contributing to ANG II-induced decreases in body weight (Fig. 4). Of the tissues examined, ANG II-infused rats exhibited significant decreases in the relative mass of retroperitoneal white fat (RPF) (adipose tissue) and the diaphragm (skeletal muscle). The other organs examined maintained their relative mass after ANG II infusion.

Study 2. To determine whether the effect of ANG II on body weight was dose dependent, rats were administered either saline or 200, 350, or 500 ng·kg$^{-1}$·min$^{-1}$ ANG II via osmotic minipump for 7 days. Measurement of plasma ANG II levels demonstrated a significant effect of ANG II [F(3,11) = 5.9, P < 0.05; Table 1]. Measurement of blood pressure demonstrated a significant effect of ANG II dose [F(3,8) = 7.2, P < 0.05], a significant effect of time of infusion [F(3,24) = 32, P < 0.05], and a significant interaction between ANG II dose and time of infusion [F(9,24) = 2.5, P < 0.05]. Mean arterial pressure was significantly increased in rats receiving 350 ng·kg$^{-1}$·min$^{-1}$ of ANG II infusion over controls at day 3, with blood pressure increased over controls at all three doses of ANG II by day 7 of ANG II infusion (Fig. 5). Moreover, at day 7 of ANG II infusion, blood pressure increases in rats receiving 500 ng·kg$^{-1}$·min$^{-1}$ ANG II were significantly greater than those observed in rats receiving the ANG II dose of 200 ng·kg$^{-1}$·min$^{-1}$.

Examination of body weight in ANG II-infused rats demonstrated a significant interaction between ANG II dose and time of infusion [F(8,64) = 25, P < 0.01]. At the lowest dose of ANG II infused (200 ng·kg$^{-1}$·min$^{-1}$), body weight was significantly decreased from saline-infused controls at day 7 of ANG II infusion (Fig. 6A). However, at ANG II infusion doses of 350 and 500 ng·kg$^{-1}$·min$^{-1}$, body weight was significantly decreased from saline-infused controls by day 5 of ANG II infusion and remained lower throughout the remainder of the experimental protocol. The time course for the effect of ANG II infusion on body weight (Fig. 6A) and blood pressure (Fig. 5) illustrates that these two variables did not change in parallel.

There was no significant effect of ANG II dose on food intake; however, there was a significant effect of the time of ANG II infusion on food intake [F(2,56) = 12.3, P < 0.01]. Reductions in food intake were evident at all three doses of ANG II infused 1 day after minipump implantation and returned to control levels by 7 days of ANG II infusion (Fig. 6B). The time course for the return of food intake to normal levels was dependent on the ANG II infusion dose. Water intake was not significantly altered by ANG II infusion (Fig. 6C). Examina-

Table 1. Plasma ANG II levels in rats from study 2

<table>
<thead>
<tr>
<th>ANG II, ng·kg$^{-1}$·min$^{-1}$</th>
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<tr>
<td>Saline</td>
<td>18.3±1.9</td>
<td>27.3±5.7</td>
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Fig. 4. Effect of ANG II infusion (175 ng·kg$^{-1}$·min$^{-1}$) on organ mass. In study 1, rats were infused with saline (open bars) or ANG II (hatched bars) for 14 days. Organs were removed at 14 days and normalized as a percentage of body weight. ISBAT, interscapular brown adipose tissue; EF, epididymal fat; RPF, retroperitoneal fat; LV, left ventricle; Diaph, diaphragm. Mass of RPF and diaphragm was decreased in ANG II-infused rats compared with saline controls. Values are means ± SE of 3/group. *Significantly different (P < 0.05) from saline control.
tion of the relative mass (%body weight) of organs removed from ANG II- and saline-infused rats demonstrated a site-specific decrease in the mass of RPF (Fig. 7) that was dependent on ANG II dose. Interestingly, measurement of plasma leptin levels at day 7 demonstrated a significant between-group effect of ANG II [\(F(3,11) = 5.4, P < 0.05\)] and a decrease in plasma leptin levels at the ANG II dose of 500 ng·kg\(^{-1}\)·min\(^{-1}\) (Fig. 8).

**Study 3.** The purpose of this study was to determine whether the effects of ANG II on body weight were independent of ANG II-induced increases in blood pressure. In study 3, ANG II was infused at a dose of 350 ng·kg\(^{-1}\)·min\(^{-1}\) for a period of 7 days. This dose of ANG II was chosen on the basis of results from study 2 demonstrating maximal effects of ANG II on body weight at an infusion dose of 350 ng·kg\(^{-1}\)·min\(^{-1}\). The vasodilator hydralazine was administered (10 mg/kg) in the drinking water of ANG II-infused and saline-infused rats for 3 days before minipump implantation and for the period corresponding to ANG II infusion. Measurement of systolic pressure demonstrated a significant effect of ANG II infusion [\(F(1,12) = 14.7, P < 0.01\)] and hydralazine treatment [\(F(1,12) = 6.1, P < 0.05\)] and a significant interaction between ANG II infusion and hydralazine treatment [\(F(1,12) = 5.4, P < 0.05\)]. Blood pressure was significantly increased in ANG II-infused rats compared with saline-infused controls by day 2 and throughout the remainder of the experimental protocol (Fig. 9). In contrast, blood pressure did not increase in ANG II-infused rats treated with hydralazine.

Examination of body weight demonstrated a significant effect of time of infusion [\(F(7,84) = 5.3, P < 0.01\)] and a significant interaction between ANG II and time of infusion [\(F(7,84) = 26.4, P < 0.01\)]. Both groups of saline-infused rats (with or without hydralazine) increased their body weight over the 7-day experimental protocol (Fig. 10A). In contrast, ANG II-infused rats did not change in body weight over the 7-day protocol. Body weight of ANG II-infused rats was significantly decreased from saline-infused controls beginning at day 5 of ANG II infusion. Interestingly, ANG II-infused rats receiving hydralazine exhibited reductions in body weight over the 7-day experimental protocol. Beginning at day 3 of ANG II infusion, body weights of hydralazine-treated rats infused with ANG II were significantly less than controls. Moreover, beginning at day 4, the body weights of ANG II-infused rats receiving hydralazine were significantly less than ANG II-infused rats. Thus, despite elimination of ANG II-induced increases in blood pressure with hydralazine treatment, the effect of ANG II infusion.
ANG II on body weight was maintained in hydralazine-treated rats. In study 3, there was a significant effect of ANG II on food intake $[F(1,12) = 29.8, P < 0.01]$ and a significant effect of time of ANG II infusion $[F(1,12) = 10.2, P < 0.01]$. Beginning at day 1 of ANG II infusion, food intake was significantly decreased in ANG II-infused rats with or without hydralazine compared with saline controls (Fig. 10B). In contrast, at a dose of 350 ng·kg$^{-1}$·min$^{-1}$ of ANG II infusion, water intake did not significantly increase (Fig. 10C). In ANG II-infused rats treated with hydralazine, water intake increased by day 1 of infusion and throughout the remainder of the experimental protocol.

Infusion of ANG II at 350 ng·kg$^{-1}$·min$^{-1}$ resulted in an increase in the relative mass of the left ventricle and a decrease in the relative mass of RPF (Fig. 11). Alterations in the mass of each of these organs were not reversed with normalization of blood pressure in hydralazine-treated rats. Plasma leptin levels were significantly decreased in ANG II-infused rats compared with saline controls (Fig. 12). However, in ANG II-infused rats treated with hydralazine, plasma leptin levels were diminished but not significantly different from controls (with or without hydralazine) or ANG II-infused rats. Thus treatment with hydralazine resulted in a partial reversal of ANG II-induced decreases in plasma leptin levels.

DISCUSSION

This study clearly demonstrates that ANG II infusion dose dependently alters the rate of weight gain and decreases body weight through pressor-independent
mechanisms. Mechanisms defined in the present study that contribute to the effect of ANG II on body weight include an increase in surface body temperature (energy expenditure), transient alterations in food intake, and alterations in plasma leptin levels. In hydralazine-treated rats with normalized blood pressure, infusion of ANG II resulted in marked reductions in body weight, demonstrating that the effect of ANG II on body weight was independent of blood pressure. With the use of infusion doses of ANG II that gradually increased blood pressure and did not elevate plasma ANG II levels, ANG II infusion was associated with a total elimination of weight gain.

Infusion of ANG II to rats has been studied extensively as a model for human renovascular and high-renin hypertension (1, 23, 34). Models of ANG II infusion have been classified as "pressor" and "subpressor," referring to the direct vasoconstrictor effects of ANG II to elicit immediate increases in blood pressure vs. slower mediated effects of ANG II at doses that do not directly influence blood pressure (34). Results from the present dose-response studies for ANG II infusion demonstrate that, at doses of ANG II infusion classified previously in the literature as pressor (>200 ng·kg⁻¹·min⁻¹) and subpressor (<200 ng·kg⁻¹·min⁻¹) (34), ANG II infusion resulted in a total elimination of weight gain and a decrease in body weight compared with saline-infused controls.
Results from study 1 demonstrate that, after infusion of ANG II at a dose of 175 ng·kg⁻¹·min⁻¹ for 14 days, plasma ANG II levels were not elevated. Plasma ANG II levels measured in control rats in the present study are in agreement with literature values for rat plasma ANG II, ranging from 8 to 30 pg/ml (31). Previous studies suggest that at infusion doses of 200 ng·kg⁻¹·min⁻¹, plasma ANG II levels increased threefold; however, elevations in plasma ANG II levels were not statistically significant because of a large variability (61% coefficient of variation) in measurements (15).

In the present study, measurements of ANG II levels in rat plasma were not associated with marked variability (controls: 4–10%; ANG II infused: 10–20% coefficient of variation). The threshold dose of ANG II infusion resulting in an increase in plasma ANG II levels in the present study was 350 ng·kg⁻¹·min⁻¹. At doses of ANG II infusion <200 ng·kg⁻¹·min⁻¹, alterations in systemic ANG II levels were not evident and are suggested to represent the high end of physiological ANG II levels.

Measurement of plasma ANG II levels in ANG II-infused rats in the present study demonstrated a threshold and sixfold increase at the two highest doses of ANG II infusion (350 and 500 ng·kg⁻¹·min⁻¹, respectively). Previous investigators have demonstrated a sevenfold increase in plasma ANG II levels in patients with human heart failure (26). Increases in plasma ANG II levels in the rat model used in the present study (0, 3-, and 6-fold) are below the reported multiples of increase (7-fold) in systemic renin-angiotensin system activation in human heart failure (26, 35). Thus alterations in body weight were evident in ANG II-infused rats at doses resulting in minimal elevations in plasma ANG II levels. The significance of the observed effects of low-dose ANG II infusion on body weight may also relate to human obesity, in which plasma volume expansion is typical with suppressed activity of the renin-angiotensin system (17, 19).

Previous investigators have demonstrated that subcutaneous ANG II infusion at a dose of 200 ng·kg⁻¹·min⁻¹ resulted in an increase in blood pressure within 1 day postinfusion (15). At a subcutaneous ANG II infusion dose of 76 ng·kg⁻¹·min⁻¹, systolic blood pressure increased by day 2 of ANG II infusion (21). In agreement with previous studies, results from this study demonstrate that infusion of ANG II at a dose of 175 ng·kg⁻¹·min⁻¹ resulted in an initial transient increase in systolic pressure at days 1 and 3 of infusion. In contrast to previous reports and results from the present study, at doses of 280 (11) and 200 ng·kg⁻¹·min⁻¹ (18), administered intraperitoneally, systolic blood pressure did not increase after 7 days of ANG II infusion, suggesting that these ANG II doses were subpressor. Results from the present study demonstrate dose-dependent effects of ANG II infusion on systolic blood pressure that were influenced by the time of infusion. However, all doses of ANG II infusion used in the present study resulted in an early increase in systolic pressure. Thus distinctions between pressor and subpressor doses of ANG II infusion in the present study were not readily apparent.

At low-dose ANG II infusion in study 1, water intake increased. In agreement with these results, previous studies demonstrate dipsogenic effects of systemically administered ANG II (37). Interestingly, in the present study, high ANG II infusion doses (≥175 ng·kg⁻¹·min⁻¹) that significantly elevated plasma ANG II levels did not result in an increase in water intake. In contrast to results from the present study, previous investigators have suggested that the threshold for the dipsogenic effect of acutely administered ANG II is ≥200 pg of ANG II per milliliter of plasma (22). Interestingly, previous investigators have shown that, after repeated intracerebroventricular administration of ANG II, tachyphylaxis to the dipsogenic effect of ANG II developed (30). On the basis of results from previous studies, a lack of dipsogenic response to chronic high-dose ANG II infusion in the present study may have resulted from tachyphylaxis or desensitization of the ANG II receptor involved in the dipsogenic effects of ANG II (30, 38).

Previous investigators demonstrated that at a dose of ANG II infusion approximately threefold greater than that used in study 1 (175 ng·kg⁻¹·min⁻¹), body weight was decreased from baseline starting values after 14 days of infusion (3). Results from study 1 extend previous findings by demonstrating that low doses of ANG II infusion classified at the threshold level for direct pressor effects markedly affected the rate of weight gain and body weight. In agreement with previous studies (3), results from this study demonstrate that higher pressor doses of ANG II result in a loss of body weight from baseline starting values. Throughout the present studies, the time course for increases in blood pressure in ANG II-infused rats did not parallel that for the effects of ANG II on body weight. Typically, increases in blood pressure were manifested 3–5 days before alterations in body weight. The time delay between blood pressure increases and elimination of weight gain after ANG II infusion suggests that these two variables are independent. Alternatively, the effect of ANG II to regulate body weight may be indirectly related to elevations in blood pressure with a time-lag delay.

Further studies using the vasodilator hydralazine demonstrated that the effect of ANG II on body weight was independent of blood pressure. These results are in agreement with previous studies demonstrating that decreases in body weight in high-dose ANG II-infused rats (500 ng·kg⁻¹·min⁻¹) were independent of elevations in blood pressure (3). Interestingly, the effect of ANG II to decrease body weight was augmented in hydralazine-treated rats. Potential mechanisms for augmentation of ANG II regulation of body weight include reflex increases in sympathetic neurotransmission in hydralazine-treated rats in response to decreased peripheral vascular resistance. Hydralazine-mediated increases in sympathetic neurotransmission would potentially increase peripheral metabolism and elevate systemic ANG II production (increased kidney-derived renin release).

The present study utilized the noninvasive method of thermal IR imaging for the regional determination of
surface temperature as an index of energy expenditure. Previous investigators have demonstrated the ability of IR thermography to detect changes in mean body surface temperature in postsurgical patients receiving total parenteral nutrition or in healthy subjects in the fasting state or after meal ingestion (33). Results from the present study demonstrate that after chronic low-dose ANG II infusion, tail surface temperature increases, suggesting that heat dissipation mechanisms were activated. In agreement with these results, previous investigators have demonstrated that acute high-dose ANG II injection resulted in an increase in tail skin temperature (36). In addition to alterations in tail temperature, results from this study demonstrate an increase in abdominal/thorax surface temperature after chronic ANG II infusion.

A variety of evidence demonstrates that ANG II facilitates the sympathetic nervous system (40). Moreover, the sympathetic nervous system is important in the control of peripheral lipid metabolism. Previous investigators chronically measured plasma norepinephrine (NE) levels in rats infused with ANG II (150 ng·kg\(^{-1}\)·min\(^{-1}\)) and demonstrated that plasma NE levels increased by days 4–6 of infusion (16). Plasma catecholamine measurements were not performed in the present study. Thus it is unclear whether the effect of ANG II to decrease body weight and increase surface temperature was mediated indirectly through activation of the sympathetic nervous system. Future studies will determine the role of the sympathetic nervous system in the metabolic effects of ANG II.

Results from this study do not support a role for alterations in food intake as the primary mechanism for the effect of ANG II on body weight. Previous investigators demonstrated that pair feeding control rats to food intake levels of ANG II-infused rats (500 ng·kg\(^{-1}\)·min\(^{-1}\)) resulted in similar levels of body weight reduction (3), suggesting that decreased food intake contributes to ANG II regulation of body weight. In the present study, high-dose ANG II infusion (>350 ng·kg\(^{-1}\)·min\(^{-1}\)) resulted in a time-dependent reduction of food intake. Initial reductions in food intake in the present study and in previous studies (3) may represent effects of ANG II related to initial pressor-mediated increases in blood pressure and general animal malaise. However, at low doses of ANG II infusion, as in study 1, the effect of ANG II on weight gain and body weight occurred in the absence of significant reductions in food intake.

Assessment of relative organ mass in the present study demonstrated a preferential effect of ANG II infusion to reduce white adipose tissue mass. Retroperitoneal white adipose tissue was significantly reduced in all of the studies performed. In contrast, other organs examined maintained their relative mass after ANG II infusion, with the exception of the diaphragm (decreased) and left ventricle (increased). The relatively specific effects of ANG II to decrease the mass of retroperitoneal white adipose tissue suggest that effects of ANG II on weight gain and body weight may arise from augmented lipid metabolism. However, in the present study, the epididymal white fat pad did not exhibit reductions in mass after ANG II infusion. These results suggest site-specific alterations in adipose lipid metabolism and mass from ANG II infusion.

The cloning of the ob/ob gene and the identification of leptin have greatly expanded the field of obesity research (39). Biological effects of adipose-derived leptin include a decrease in food intake and an increase in energy expenditure (5, 27). Increases in energy expenditure after leptin administration are associated with elevations in NE turnover and enhanced brown adipose thermogenesis (10). In a feedback endocrine regulatory loop, the sympathetic nervous system has been demonstrated to negatively modulate leptin gene expression in white adipose tissue (20). In the present study, measurement of plasma leptin levels after chronic ANG II infusion demonstrated that high-dose ANG II resulted in a decrease in plasma leptin. A limitation of the present study is that chronic measurements of plasma leptin were not obtained during ANG II infusion; thus it is unclear whether suppressed leptin levels may represent a compensatory response to chronic ANG II infusion, potentially mediated through sympathetic nervous system negative feedback. Alternatively, decreases in plasma leptin levels after chronic ANG II infusion may arise from semistarved states of rats (decreased food intake) or reductions in the mass of white adipose tissue. Future studies will determine the role of leptin in ANG II regulation of body weight. Regardless, these studies are the first to demonstrate that ANG II influences plasma leptin secretion.

In summary, results from this study demonstrate that ANG II regulates body weight through pressor-independent mechanisms in a dose-dependent manner. Furthermore, mechanisms contributing to ANG II regulation of body weight include alterations in plasma leptin, mobilization of fat mass, and increased energy expenditure. These findings are relevant to disease states associated with heightened (congestive heart failure) or diminished (obesity) activity of the renin-angiotensin system. Moreover, results from this study support a functional role for ANG II production in adipose tissue and strengthen the physiological significance of an adipose renin-angiotensin system.

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