Angiotensin II regulates oxygen consumption

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Cassis, Lisa, Marc Helton, Vicki English, and Gerome Burke. Angiotensin II regulates oxygen consumption. Am J Physiol Regulatory Integrative Comp Physiol 282: R445–R453, 2002; 10.1152/ajpregu.00261.2001.—Previous studies demonstrated that angiotensin II (ANG II) decreases body weight. This study examined whether ANG II regulates body weight through energy expenditure. Acute ANG II administration decreased oxygen consumption. To determine whether this effect was maintained, rats were infused with ANG II or saline for 14 days. Oxygen consumption was transiently decreased on day 1 of ANG II infusion; however, body weight and food intake were reduced for 14 days. In pair-feeding studies, reductions in food intake accounted for 63% of the effect of ANG II on body weight but did not influence systolic pressure, water intake, or oxygen consumption. With 28 days of ANG II infusion, differences in body weight between ANG II and control rats were of greater magnitude. An initial decrease in oxygen consumption was followed by a rebound increase. Co-administration of losartan prevented the effect of ANG II on body weight, food intake, blood pressure, and water intake. However, losartan only partially prevented ANG II reductions in oxygen consumption. These results demonstrate that ANG II transiently decreases oxygen consumption through mechanisms unrelated to food intake. With chronic ANG II exposure, energy expenditure may contribute to sustained reductions in body weight; energy expenditure.

ALL THE COMPONENTS of the renin-angiotensin system have been identified in adipose tissue (for review see Ref. 13), including angiotensinogen (5, 7, 8, 24), renin (18, 25), angiotensin-converting enzyme (11, 18, 25), and angiotensin II (ANG II) receptors (4, 10). Angiotensinogen mRNA expression in adipose tissue is regulated by a variety of factors, including insulin (20), fatty acids (23), glucocorticoids (1), and nutritional status (16). Processing enzymes important in ANG II formation have been localized to adipose tissue (24, 25), and ANG II peptides have been detected in adipocyte cell lines (24) and adipose tissue slices (3, 25). ANG II has been demonstrated to stimulate the release of prostacyclin from mature fat cells (12). Moreover, ANG II regulates enzymes important in fatty acid synthesis and metabolism (19). Collectively, these results have suggested the existence of a local renin-angiotensin system in adipose tissue (13), with the potential for adipocytes to produce and respond to ANG II. However, the functional significance of an adipose renin-angiotensin system and the potential physiological effects of ANG II at adipose tissue remain largely undefined.

As an approach to determine the physiological effects of ANG II at adipose tissue, previous studies in our laboratory examined the effect of chronic infusion of ANG II on the regulation of body weight in rats (6). Results from these studies demonstrated that chronic ANG II infusion resulted in a dose-dependent reduction in body weight that was independent of blood pressure (6). The ability of ANG II to decrease body weight was associated with reductions in food intake (2, 6); however, pair-feeding studies suggested that other mechanisms contribute to the effect of ANG II to regulate body weight (2). ANG II decreased body weight at doses that did not significantly elevate plasma ANG II concentration (6); thus it is conceivable that local adipose ANG II production could mediate this effect. The functional significance of this effect of ANG II was suggested to relate to disease states with heightened activity of the renin-angiotensin system, such as human heart failure or cirrhosis, where excessive peripheral metabolism and body wasting are significant clinical concerns.

Recent studies in our laboratory suggested that heightened sympathetic drive may contribute to the ability of ANG II to decrease body weight (14). After chronic ANG II infusion, plasma norepinephrine (NE) concentration was increased (14), catecholamine turnover was elevated in adipose tissue (unpublished observations), and NE release from slices of brown adipose tissue was increased (14). Collectively, these results suggested that ANG II may increase sympathetic drive to metabolically relevant tissues, including adipose tissue or skeletal muscle, and thereby increase peripheral energy expenditure. A well-known measure of peripheral energy expenditure is the determination of whole animal oxygen consumption using indirect calorimetry. The purpose of this study was to determine the effect of acute vs. chronic ANG II administration on whole body oxygen consumption as a measure of peripheral energy expenditure. We hypothesized...
that ANG II-mediated reductions in body weight resulted from stimulation of sympathetic drive to metabolically relevant tissues, ultimately contributing to elevations in peripheral energy expenditure. These results would support a metabolic effect of ANG II, potentially related to elevated peripheral energy expenditure in disease states with heightened activity of the renin-angiotensin system. Moreover, definition of the metabolic effect of ANG II would increase our understanding of the relevance of adipose ANG II formation.

METHODS

Animals. Male Sprague-Dawley rats (350–400 g; Harlan Sprague Dawley, Indianapolis, IN) were housed two per cage in an approved animal facility for 1 wk before use under a 12:12-h light-dark cycle and were given free access to food and water. During each experimental protocol, the rats were housed individually in cages for the daily (10 AM) measurement of body weight and food and water intake. For pair-feeding studies, a third group of rats was infused with saline and pair fed with ANG II-infused rats. All studies were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

Drug treatment. For acute drug treatment, rats were injected with ANG II (20 or 200 μg/kg ip), isoproterenol (2.5 or 25 μg/kg ip), or an equivalent volume of vehicle (sterile saline). For chronic ANG II infusion, rats were anesthetized with diethyl ether and shaved in the interscapular region, according to the manufacturer’s instructions before implantation to ensure immediate subcutaneous drug delivery. The rats were maintained with chronic ANG II exposure and losartan and were primed with ANG II, losartan, or pair feeding as between-group factors and time as a within-group repeated measure. Post hoc analysis was performed using Tukey’s multiple comparison test with significance at P < 0.05.

RESULTS

Acute administration of ANG II decreases oxygen consumption. To validate the oxygen consumption apparatus, rats (n = 5/dose) were treated with the β-receptor agonist isoproterenol at 2.5 or 25 μg/kg ip. The time course for oxygen consumption in rats for 120 min before and after injection of isoproterenol are illustrated in Fig. 1. At 2.5 μg/kg, isoproterenol did not significantly influence oxygen consumption compared with pretreatment values (Fig. 1). At 25 μg/kg, isoproterenol resulted in a significant increase in oxygen consumption from 80 to 120 min after injection, demonstrating the sensitivity of the apparatus.

In separate studies, the effect of a single dose of ANG II (20 or 200 μg/kg ip, n = 5/dose) on oxygen consumption was determined. Oxygen consumption was measured for 120 min before and after injection of ANG II (Fig. 1). At 20 μg/kg, ANG II resulted in a shift to the right in the time course for oxygen consumption but no difference in the peak value obtained at 120 min. At 200 μg/kg, ANG II resulted in a shift to the right in the time course for oxygen consumption and a significant depression of the peak value at plateau. These results demonstrate that the acute administration of ANG II decreases oxygen consumption.

Effect of chronic administration of ANG II on oxygen consumption. The previous experiment demonstrated that acute administration of ANG II decreased oxygen consumption. To determine whether this acute effect was maintained with chronic ANG II exposure and coincided with ANG II regulation of blood pressure or body weight, we infused ANG II at 400 ng·kg⁻¹·min⁻¹ or saline for 14 days (n = 5 rats/group). Blood pressure was increased by chronic infusion of ANG II on day 7 and was maintained at a greater level than controls on day 14 (Fig. 2). Within 4 days of ANG II infusion, body weight was significantly decreased compared with control (Fig. 2). Moreover, body weight was further reduced during week 2 of ANG II infusion. Food intake decreased significantly within 3 days of ANG II infusion and remained significantly decreased compared with control over the time course of the study (Fig. 2).
In rats receiving saline, peak oxygen consumption was similar over 14 days of infusion (Fig. 3). In contrast, a significant decrease in peak oxygen consumption induced by ANG II on day 1 was not maintained over the time course of the study. The data in Fig. 2B illustrate the oxygen consumption over the time that rats were placed in the metabolic chamber for ANG II- and saline-infused rats on day 2 of the study. Peak oxygen consumption was calculated from the plateau portion of the curves and was significantly decreased in ANG II-infused rats compared with control. The slope of the curve for ANG II-infused rats was shifted to the left.

Because results from these studies demonstrated that chronic ANG II infusion decreased food intake, in follow-up experiments, we examined an additional group of saline-infused rats that were pair fed to the food intake of rats receiving ANG II (n = 5 rats/group). Systolic blood pressure in ANG II-infused rats was significantly increased compared with baseline (data not shown) and compared with pair-fed rats (94 ± 6 mmHg for saline-infused pair-fed rats and 155 ± 16 mmHg for ANG II-infused rats, P < 0.05). Food intake was significantly decreased in both groups of rats compared with baseline values (day 0) within 2 days and was maintained at a similar level in both groups through day 14 (Fig. 4A). Body weight was significantly decreased in ANG II-infused rats compared with baseline (day 0) from day 5 through day 14 (Fig. 4B). In pair-fed rats, body weight reductions paralleled those in ANG II-infused rats from day 1 to day 4. Thereafter, the two groups diverged, and body weight decreased further in ANG II-infused rats than in pair-fed controls. Water intake increased significantly in ANG II-infused rats compared with baseline but was not influenced by pair feeding (Fig. 4C). Peak oxygen consumption was significantly decreased in rats chronically infused with ANG II from day 1 to day 7 (Fig. 5). In contrast, pair-fed rats did not exhibit a change in peak oxygen consumption over the 14-day period of the study.

To determine whether the effects of ANG II to regulate blood pressure, body weight, food intake, and oxygen consumption were maintained beyond 14 days, additional studies examined the effect of chronic infusion of saline or ANG II (n = 6/group) for 28 days. Because of the limits of solubility of ANG II in the minipump reservoir volume, the dose of ANG II for these 28-day studies was reduced to 260 ng·kg⁻¹·min⁻¹. Systolic blood pressure was significantly increased in ANG II-infused rats compared with saline controls (137 ± 6 and 171 ± 14 mmHg for saline- and ANG II-infused rats, respectively, P < 0.05). Body weight decreased compared with starting body weight and compared with saline controls; on day 28, a 100-g (22%) reduction in body weight was demonstrated in ANG II-infused rats compared with saline controls (Fig. 6A). Food intake decreased by 36% within 2 days and remained reduced compared with saline controls over the 28-day study (Fig. 6B). Water intake was increased throughout the duration of ANG II exposure compared with saline controls (Fig. 6C). Peak oxygen consumption decreased initially in ANG II-infused rats from day 2 to day 10 compared with starting values and saline controls, increased transiently from day 16 to day 18 of ANG II exposure, and thereafter was not different from saline controls (Fig. 7).

To determine whether the angiotensin type 1 (AT₁) receptor mediates the effect of ANG II on body weight,
food intake, and oxygen consumption, the effect of losartan on these ANG II-induced responses was examined. Saline, losartan (30 mg·kg⁻¹·day⁻¹), ANG II (400 ng·kg⁻¹·min⁻¹), or ANG II + losartan (n = 5 rats/group) was administered to four groups of rats for 7 days. Systolic blood pressure was significantly increased in ANG II-infused rats compared with controls (86 ± 6 and 137 ± 12 mmHg in saline- and ANG II-infused rats, respectively, P < 0.05). Administration of losartan alone had no significant effect on blood pressure (data not shown); however, losartan administration prevented ANG II-induced increases in systolic pressure (100 ± 10 mmHg in rats treated with ANG II + losartan). ANG II + losartan totally prevented ANG II-induced reductions in body weight (Fig. 8A), food intake (Fig. 8B), and water intake (Fig. 8C). These results demonstrate that the AT₁ receptor mediates these effects of ANG II. Chronic infusion of ANG II reduced peak oxygen consumption at days 2 and 7 compared with saline control and baseline (day 0) values (Fig. 9). ANG II + losartan prevented ANG II-induced reductions in oxygen consumption on day 2. However, losartan administration only partially prevented ANG II-induced reductions in oxygen consumption on day 7.

**DISCUSSION**

The purpose of this study was to determine whether ANG II regulation of body weight was associated with regulation of oxygen consumption as a measure of peripheral energy expenditure. Results from this study demonstrate that acute and chronic exposure to ANG II transiently decreased oxygen consumption. How-

Fig. 2. Effect of chronic ANG II infusion on blood pressure, body weight, and food intake. ANG II (400 ng·kg⁻¹·min⁻¹) or saline was infused for 14 days. Systolic blood pressure was increased in ANG II-infused rats compared with baseline values and compared with saline control rats on days 7 and 14. Body weight was reduced in ANG II-infused rats beginning on day 4 compared with saline controls. Food intake was reduced beginning on day 2 in ANG II-infused rats compared with controls. Values are means ± SE from 5 rats/group. *Significantly different from control (P < 0.05).

Fig. 3. Chronic infusion of ANG II transiently reduces O₂ consumption. Rats were infused with ANG II (400 ng·kg⁻¹·min⁻¹) or saline for 14 days. O₂ consumption was measured as described in METHODS. A: peak O₂ consumption was transiently decreased in ANG II-infused rats compared with baseline values and compared with saline controls on day 1. B: O₂ consumption over the time rats were placed in the metabolic chamber. Results are for rats from each group on day 2 and demonstrate that ANG II infusion decreased peak O₂ consumption and shifted the curve to the left. Plateau value was used for peak O₂ consumption measurements in A. Values are means ± SE from 5 rats/group.
ever, initial reductions in oxygen consumption from chronic ANG II exposure were not maintained, and oxygen consumption returned to normal, despite a sustained elevation in blood pressure and reduction in food intake. Moreover, results from pair-feeding studies demonstrated that the observed decrease in oxygen consumption after ANG II exposure did not result from reductions in food intake. However, reductions in food intake did contribute significantly to ANG II-induced reductions in body weight. Finally, although ANG II regulation of food intake and body weight occurred through actions at the AT₁ receptor, antagonism at the AT₁ receptor only partially reversed the effect of ANG II on oxygen consumption. These results suggest that 1) ANG II regulates body weight initially through AT₁-mediated reductions in food intake, 2) mechanisms unrelated to food intake may contribute to initial reductions in peripheral energy expenditure after ANG II exposure, and 3) with more chronic ANG II exposure, effects of ANG II on energy expenditure may contribute to further reductions in body weight.

The physiological effects of chronic ANG II exposure on the cardiovascular system are well known and include direct pressor effects at vascular smooth muscle, an increase in the synthesis and secretion of aldosterone, stimulation of sympathetic neurotransmission, and the induction of cardiac and vascular hypertrophy. Several of these and other effects of ANG II may have an impact on peripheral energy expenditure. First, the direct vasoconstrictor effect of ANG II at AT₁ receptors on vascular smooth muscle cells would predictably reduce blood flow in the periphery and, thereby, change the rate of supply of nutrients to muscle and removal of metabolic products (9, 22). After acute ANG II infusion, previous investigators demonstrated that microvascular blood flow was reduced in the perfused rat hindlimb (9, 22). However, evidence suggested that vasoconstriction produced by low-dose ANG II actually increased basal oxygen uptake in skeletal muscle (9, 22). On the basis of previous findings in the literature, we suggest that vasoconstriction in response to ANG II did not contribute to the observed transient decrease in oxygen consumption in this study. Moreover, despite sustained increases in blood pressure with ANG II exposure, oxygen consumption returned to pretreatment values, suggesting that ANG II regulation of oxygen consumption is not totally dependent on pressure effects of the peptide. Finally, given that the infused doses of ANG II were sufficient for direct pressor effects and would therefore elicit baroreceptor-mediated reductions in sympathetic outflow, even a normal level of ANG II regulation of O₂ consumption does not result from reductions in food intake. In the same rats described in Fig. 4, O₂ consumption was measured as described in METHODS. Pair feeding to the food intake level of ANG II-infused rats had no effect on O₂ consumption. In rats infused with ANG II, O₂ consumption was reduced during week 1 of infusion and returned to baseline values during week 2. Values are means ± SE from 5 rats/group. *Significantly different from pair-fed rats (P < 0.05).

Fig. 4. Effect of pair feeding on ANG II regulation of body weight in rats infused with ANG II (400 ng·kg⁻¹·min⁻¹) and saline-infused rats pair fed to the food intake of rats receiving ANG II. Food intake was decreased in ANG II-infused rats by day 1 and gradually returned toward baseline (day 0) values; food intake was the same in pair-fed and ANG II-infused rats. Body weight decreased in both groups of rats to the same extent for 1 wk; thereafter, the 2 groups diverged, and body weight was lower in ANG II-infused rats than in pair-fed rats. Water intake was increased in ANG II-infused rats; pair feeding had no effect on water intake. Values are means ± SE from 5 rats/group. *Significantly different from pair-fed rats (P < 0.05).

Fig. 5. ANG II regulation of O₂ consumption does not result from reductions in food intake. In the same rats described in Fig. 4, O₂ consumption was measured as described in METHODS. Pair feeding to the food intake level of ANG II-infused rats had no effect on O₂ consumption. In rats infused with ANG II, O₂ consumption was reduced during week 1 of infusion and returned to baseline values during week 2. Values are means ± SE from 5 rats/group. *Significantly different from pair-fed rats (P < 0.05).
of oxygen consumption in ANG II-infused rats may have been inappropriately high.

A second mechanism potentially contributing to the ability of ANG II to regulate oxygen consumption includes regulation of hypothalamic thermoregulatory regions in the brain. A reduction in oxygen consumption, coupled with a decrease in colonic temperature and an increase in tail skin temperature, was previously reported after a similar dose of acutely administered ANG II (17, 26). The observed reduction in oxygen consumption after acute ANG II administration was suggested to result from a hypothalamic effect of the peptide to increase heat dissipation and reduce heat production (17, 26). Our results agree with these previous findings and demonstrate that acute administration of ANG II reduced oxygen consumption. Additionally, results from this study extend previous findings by demonstrating that these acute responses to ANG II are evident during week 1 of exposure to the peptide. However, reductions in oxygen consumption were not maintained with more chronic ANG II exposure. In contrast, transient increases in oxygen consumption were observed with more chronic ANG II infusion. These results suggest that the effects of the peptide in thermoregulatory regions of the hypothalamus on acute exposure may be overridden or lost with chronic ANG II exposure. However, chronic ANG II exposure continued to increase water intake, an additional effect of ANG II mediated through the hypothalamus.

A third mechanism potentially contributing to the ability of ANG II to regulate oxygen consumption includes the observed effects of chronic ANG II infusion to decrease food intake. Previous investigators have demonstrated that pair feeding of rats to food intake levels of ANG II-infused rats accounted for ~70% of the body weight-reducing effects of the peptide (2); however, oxygen consumption was not measured in previous studies. The authors suggested that the remaining 30% reduction in body weight may arise from stimulation of sympathetic nervous system activity by ANG II and resultant increases in basal metabolism. In agreement with previous findings, our results demonstrate that reductions in food intake accounted for 63% of the decrease in body weight after 14 days of ANG II infusion. It is well documented that, under conditions of prolonged fasting or food restriction, metabolic rate may eventually decrease to compensate partially for the energy deficit (15). Thus ANG II-mediated reductions in food intake could conceivably contribute to any observed decrease in oxygen consumption. In agreement, in this study the initial reduction in oxygen consumption with chronic ANG II exposure occurred coincidentally with reductions in food intake. However,
despite the sustained reduction in food intake with chronic ANG II exposure, oxygen consumption returned to normal values. More importantly, in rats pair fed to food intake levels of ANG II-infused rats, oxygen consumption did not change from baseline, demonstrating that ANG II-mediated reductions in food intake are not responsible for the observed decrease in oxygen consumption.

A fourth mechanism potentially contributing to the effects of ANG II to regulate oxygen consumption includes stimulation of the sympathetic nervous system. A well-documented effect of ANG II is an increase in central nervous system sympathetic outflow as well as stimulation of NE release from peripheral sympathetic nerve terminals (3, 14). Our previous results demonstrated that ANG II can increase NE release from sympathetic nerve terminals innervating rat brown adipose tissue (3) and that these effects of ANG II are increased on chronic ANG II exposure (14). Moreover, previous studies have demonstrated that chronic ANG II infusion increases catecholamine turnover as an index of sympathetic activity in a variety of tissues (21), including adipose tissue (unpublished observations). Stimulation of sympathetic outflow to adipose tissue would increase lipolysis and provide an efficient fuel for thermogenesis and, thereby, could potentially increase oxygen consumption. However, results from this study demonstrate that ANG II infusion initially decreased, rather than increased, peripheral energy expenditure. In contrast, with longer-term ANG II exposure, oxygen consumption transiently increased and then returned to normal values, despite sustained increases in blood pressure and reductions in food intake. Moreover, when ANG II was infused for 28 days, further reductions in body weight during week 2 of infusion coincided with the rebound increase in oxygen consumption. We suggest that the initial drop in oxygen consumption with ANG II infusion does not relate to the ability of the peptide to decrease body weight; however, with chronic ANG II exposure, increases in sympathetic outflow with resultant effects on energy expenditure may contribute to sustained ANG II-mediated reductions in body weight.

A fifth mechanism whereby ANG II may influence oxygen consumption relates to the effect of the peptide on adipocyte function. Previous investigators have demonstrated that ANG II, through direct or indirect...
mechanisms, can regulate factors produced by adipocytes, including prostacyclin (12) and leptin (6). The ability of ANG II to increase prostacyclin release from adipocytes has been implicated in the growth and differentiation of preadipocytes to adipocytes (11). As for leptin, previous studies in our laboratory have demonstrated a decrease in plasma leptin concentration after chronic ANG II infusion (6). In addition, previous investigators have demonstrated that ANG II can increase lipogenesis in 3T3-L1 adipocytes and human adipose cells (19), providing a greater amount of lipolytic fuel for energy expenditure. These effects of ANG II may collectively contribute to the ability of chronic ANG II exposure to regulate oxygen consumption.

In conclusion, results from this study demonstrate that acute and chronic exposure to ANG II decreases oxygen consumption. With chronic ANG II exposure, transient reductions in oxygen consumption coincided with reductions in food intake and body weight. However, pair-feeding studies demonstrated that transient reductions in oxygen consumption in response to ANG II were independent of reductions in food intake. In contrast, reductions in food intake did significantly contribute to ANG II-mediated decreases in body weight. With more chronic ANG II exposure (>2 wk), oxygen consumption returned to normal values and coincided with further reductions in body weight, suggesting that the long-term ability of the peptide to decrease body weight may relate to energy expenditure. The clinical implication of these results relates to disease states with heightened activity of the renin-angiotensin system, such as congestive heart failure, whereby elevations in systemic ANG II may contribute to the dysregulation of body weight. Moreover, the ability of elevated systemic ANG II to decrease oxygen consumption may relate to the diagnostically important and well-defined reduction in peak oxygen consumption in patients with congestive heart failure. Finally, these results suggest that local adipose ANG II production may bear physiological significance in the control of energy expenditure and resultant changes in body weight.

Perspectives

The renin-angiotensin system is a primary regulator of fluid and electrolyte balance, with aberrant activity of the system involved in the pathophysiology of hypertension and congestive heart failure. Given the cardiovascular importance of ANG II, considerable research has focused on local tissue production of the peptide. Previous studies in our laboratory have suggested the presence of local ANG II production in adipose tissue. However, the physiological significance of ANG II production by adipocytes remains undefined. In a search for an adipocyte-related function of ANG II, we used a well-known model of renovascular hypertension, namely, chronic infusion of ANG II to rats. Our results demonstrate that chronic infusion of ANG II to rats reduces body weight and that these effects of ANG II are magnified and maintained over time. In this study, we addressed whether ANG II-mediated reductions in body weight occur from an elevation in oxygen consumption as a measure of peripheral energy expenditure. Our rationale for examining energy expenditure in ANG II-treated rats was based on the observation that, in disease states associated with high circulating ANG II, such as end-stage congestive heart failure or cirrhosis, body wasting and/or cachexia are of significant clinical concern. Our results demonstrate that ANG II initially decreases oxygen consumption; however, oxygen consumption then increased above normal. Moreover, given the pressor effects and food-intake reducing properties of ANG II, even a normal level of oxygen consumption was probably inappropriately high. Although we did not define the precise mechanism of the effect of ANG II, our results demonstrate that these effects of ANG II were independent of food intake and mediated by the AT1 receptor. The broad implication of these findings is that chronic marked elevations in circulating ANG II may increase catabolism and contribute to cachexia. Future studies will determine the role of adipose-derived ANG II in the regulation of energy expenditure and body weight.

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