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Am J Physiol Endocrinol Metab 292:1280-1287, 2007. First published Jan 9, 2007;

doi:10.1152/ajpendo.00277.2006

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Angiotensin II increases adipose angiotensinogen expression

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Submitted 9 June 2006; accepted in final form 4 January 2007

Lu H, Boustany-Kari CM, Daugherty A, Cassis LA. Angiotensin II increases adipose angiotensinogen expression. *Am J Physiol Endocrinol Metab* 292: E1280–E1287, 2007. First published January 9, 2007; doi:10.1152/ajpendo.00277.2006.—In addition to the well-defined contribution of the liver, adipose tissue has been recognized as an important source of angiotensinogen (AGT). The purpose of this study was to define the angiotensin II (ANG II) receptors involved in regulation of adipose AGT and the relationship of this control to systemic AGT and/or angiotensin peptide concentrations. In LDL receptor-deficient (LDLR^{-/-}) male mice, adipose mRNA abundance of AGT was 68% of that in liver, and adipose mRNA abundance of the angiotensin type 1a (AT_{1a}) receptor (AT_{1a}R) was 38% of that in liver, whereas mRNA abundance of the angiotensin type 2 (AT₂) receptor (AT₂R) was 57% greater in adipose tissue than in liver. AGT and angiotensin peptide concentrations were decreased in plasma of AT_{1a}R-deficient (AT_{1a}R^{-/-}) mice and were paralleled by reductions in AGT expression in liver. In contrast, adipose AGT mRNA abundance was unaltered in AT_{1a}R^{-/-} mice. AT₂R^{-/-} mice exhibited elevated plasma angiotensin peptide concentrations and marked elevations in adipose AGT and AT_{1a}R mRNA abundance. Increases in adipose AGT mRNA abundance in AT₂R^{-/-} mice were abolished by losartan. In contrast, liver AGT and AT_{1a}R mRNA abundance were unaltered in AT₂R^{-/-} mice. Infusion of ANG II for 28 days into LDLR^{-/-} mice markedly increased adipose AGT and AT_{1a}R mRNA but did not alter liver AGT and AT_{1a}R mRNA. These results demonstrate that differential mRNA abundance of AT_{1a}/AT₂ receptors in adipose tissue vs. liver contributes to tissue-specific ANG II-mediated regulation of AGT. Chronic infusion of ANG II robustly stimulated AT_{1a}R and AGT mRNA abundance in adipose tissue, suggesting that adipose tissue serves as a primary contributor to the activated systemic renin-angiotensin system.

angiotensin II receptors; renin-angiotensin system; cardiovascular disease; obesity-related disorders; metabolic syndrome

THE RENIN-ANGIOTENSIN SYSTEM (RAS) is well known to regulate blood pressure and fluid homeostasis. Activation of the RAS is a common feature in patients with the metabolic syndrome (47). Angiotensinogen (AGT), the only known precursor of angiotensin II (ANG II), is produced primarily by the liver (16). However, AGT mRNA is expressed by a variety of tissues. Thus, under different physiological/pathophysiological conditions, such as the metabolic syndrome, alternative tissues may contribute to the circulating AGT concentration. We previously demonstrated a high level of AGT mRNA expression in white and brown adipocytes (9, 10, 50, 56). Moreover, expression of AGT in adipose tissue is nutritionally and hormonally regulated by obesity (3, 25, 48, 61), insulin (30), fatty acids (49), and cAMP (55). Furthermore, previous studies demonstrated that transgenic overexpression of AGT in adi-

pose tissue leads to its secretion into the circulation and rescues hypotension and leanness of AGT-deficient mice (43). These observations suggest that adipose-derived AGT can contribute to plasma AGT concentrations and may be involved in the pathogenesis of obesity-related diseases.

The RAS is considered to be a classical endocrine feedback regulatory system, exerting control over the synthesis and secretion of RAS components by end-product peptides. Well-recognized sites for endocrine feedback regulation of the RAS by ANG II include AGT in the liver and renin in the kidney. ANG II positively regulates liver AGT mRNA expression and circulating AGT concentrations (33, 44) but negatively regulates kidney renin mRNA expression and release (52). The significance of positive- and negative-feedback regulation by ANG II on RAS component expression differs across species. For example, in rats and humans, the predominant effect is to reduce further ANG II synthesis by suppression of renal synthesis and secretion of renin, the rate-limiting step in ANG II production (52). In contrast, studies in mice suggest that AGT may limit the rate of production of systemic ANG II (11, 40). Thus, in mice, ANG II may predominantly increase further peptide synthesis by increasing the concentration of circulating AGT. Feedback regulation of AGT and renin by ANG II is mediated primarily through the angiotensin type 1 (AT₁) receptor (AT₁R) (34, 38).

In contrast to endocrine feedback regulation by ANG II in tissues with well-documented roles in the synthesis of individual RAS components, less is understood regarding endocrine feedback regulation in alternative sites of expression that are recognized as important for local production of this peptide. Given that AT_{1a}Rs are expressed in mouse adipose tissue (5, 42), we propose that ANG II exerts endocrine feedback regulation of AGT synthesis in adipocytes. Previously, we showed that the RAS is increased in hypercholesterolemic LDL receptor (LDLR)-deficient (LDLR^{-/-}) mice (13). Therefore, in this study, we used LDLR^{-/-} mice to attain a lipoprotein-cholesterol distribution that was more similar to that of humans. In addition, since our previous studies suggest that AGT may limit the rate of synthesis of ANG II in C57BL/6 mice (15), we used LDLR^{-/-} mice with elevated systemic AGT to more closely mimic humans. We first compared RAS component expression of AGT, AT_{1a}R, and angiotensin type 2 (AT₂) receptors (AT₂Rs) between adipose tissue and liver. We then determined the regulation of liver vs. adipose AGT and AT_{1a}R mRNA abundance in AT_{1a}R- and AT₂R-deficient (AT_{1a}R^{-/-} and AT₂R^{-/-}) mice. Regulation of RAS expression in tissues was paralleled by measurement of plasma AGT and angiotensin peptide concentrations in mice with specific ANG II receptor

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Table 1. Oligonucleotides, cDNA sequences, amplicon length, and annealing temperature

	Primers	Amplicon Length, bp	Annealing Temp, °C
AGT			
5'	GTACAGACAGCACCCTACTT	187	60
3'	CACGTCACGGAGAAGTTGTT		
AT _{1a} R			
5'	GACCAACTCAACCCAGAAAAGC	177	60
3'	ATCACCACCAAGCTGTTTCC		
AT ₂ R			
5'	GATGGAGGGAGCTCGGAACT	143	60
3'	TTGAACTGCAGCAACTCCAATT		
18S rRNA			
5'	CTCTGTTCGGCTAGTCTCTG	151	60
3'	AATGAGCCATTGCGAGTTTC		

AGT, angiotensinogen; AT_{1a}R, angiotensin type 1a receptor; AT₂R, angiotensin type 2 receptor.

deficiencies. Finally, we examined the effect of chronic ANG II infusion on adipose vs. liver AGT and AT_{1a}R expression.

MATERIALS AND METHODS

Mice and diet. LDLR^{-/-} male mice (B6.129S7-Ldlr^{tm1Her}; stock no. 002207) and AT_{1a}R^{-/-} male mice (B6.129P2-Agr1a^{tm1Unc};

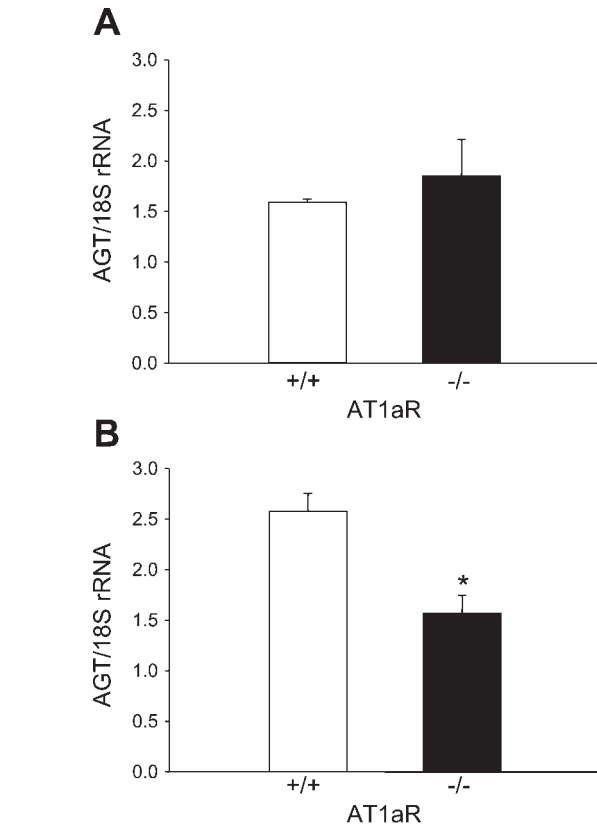
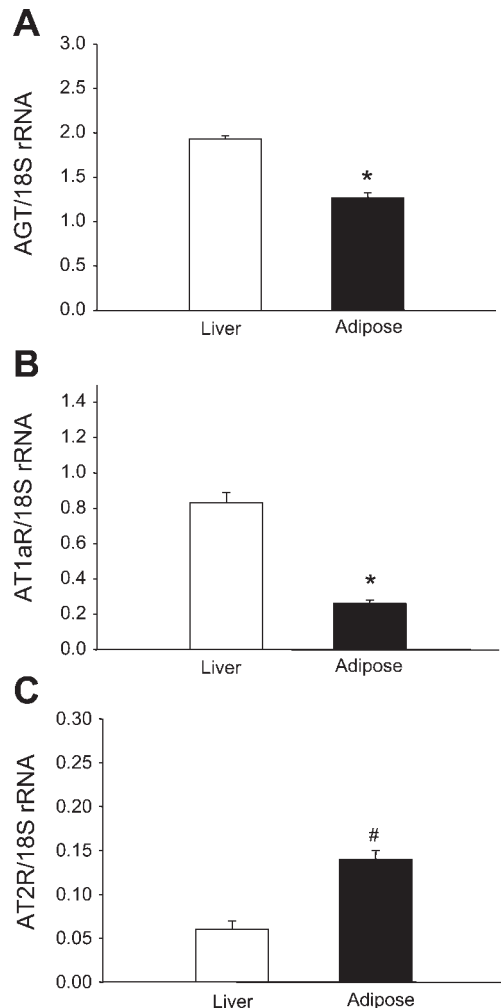


Fig. 2. Effect of AT_{1a}R deficiency on AGT mRNA abundance in adipose tissue (A) and liver (B). Adipose AGT mRNA abundance was not altered in AT_{1a}R-deficient (AT_{1a}R^{-/-}) mice. Liver AGT abundance was decreased in AT_{1a}R^{-/-} compared with wild-type mice. Data are expressed as ratio of 18S rRNA abundance for each sample. Values are means \pm SE ($n = 4$ mice/group). * $P < 0.01$.

stock no. 002682) were obtained from the Jackson Laboratory. AT₂R^{-/-} mice were a kind gift of Dr. T. Inagami (Vanderbilt University). All genotypes had been backcrossed 10 times into a C57BL/6 background. Littermates (LDLR^{-/-}, AT_{1a} \times LDLR^{-/-}, and AT₂ \times LDLR^{-/-}) were used for the present study. All mice were maintained in a barrier facility and fed normal mouse laboratory diet containing 5% fat, 20% protein, and 3.75 kcal/g digestible energy (diet no. 7012, Harlan Teklad, Madison, WI). All studies using animals were performed in accordance with American Association for Accreditation of Laboratory Animal Care "Guidelines for the Care and Use of Experimental Animals" and were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

Genotyping by PCR. For AT_{1a}R genotyping, the following primers were used: 5'-AAATGGCCCTTAACCTTCTACTG-3' (antisense) and 5'-ATTAGGAAAGGGAACAGGAAGC-3' (sense). Resultant wild-type and deficient allele bands were 650 bp and 1.1 kb, respectively. For AT₂R genotyping, the following primers were used: 5'-GTAA-GAATTTGGAGTTGCTG-3' (sense) and 5'-GGGATTCCTTCTTT-GAGAC-3' (antisense). Resultant wild-type and deficient allele bands

Fig. 1. Relative mRNA abundance of angiotensinogen (AGT, A), angiotensin type 1a (AT_{1a}) receptor (AT_{1a}R, B), and angiotensin type 2 (AT₂) receptor (AT₂R, C) in mouse liver and epididymal adipose tissue. AGT mRNA expression was higher in liver than in adipose tissue. Abundance of the AT_{1a}R was ~2-fold greater in liver than in adipose tissue. AT₂R mRNA abundance was 57% greater in adipose tissue than in liver. Data are expressed as ratio of 18S rRNA abundance for each sample. Values are means \pm SE ($n = 4$ –5 mice/group). * $P < 0.001$. # $P = 0.001$.

were 500 bp and 1.1 kb, respectively. For LDLR genotyping, the following primers were used: 5'-AGGTGAGATGACAGGAGATC-3', 5'-AGGATGACTTCCGATGCCAG-3', and 5'-GCAGTGCTC-CTCATCTGACTTG-3'. Resultant wild-type and deficient allele bands were 383 and 800 bp, respectively.

Total RNA isolation and reverse transcriptase PCR. Total RNA was extracted from mouse epididymal adipose tissue and liver using the SV total RNA isolation system (Promega, Madison, WI) according to the protocol provided by the manufacturer. Extracted RNAs were suspended in ribonuclease-free water and quantified by measurement of absorbance at 260 nm.

The abundance of mRNA in mouse adipose tissue and liver was estimated by real-time PCR using an iCycler (Bio-Rad), and the threshold cycle number was determined using iCycler software version 3.0. Real-time PCR product accumulation was monitored using the intercalating dye SYBR Green I, which exhibits a higher fluorescence on the binding of double-stranded DNA. The relative abundance of each mRNA was estimated using a standard curve constructed from serial dilutions of control cDNA. The mRNA abundance was calculated as the ratio of the gene of interest to 18S rRNA. Nontemplate control and non-RT control were used as a negative control. The real-time PCR was performed using the primers shown in Table 1.

Administration of losartan or ANG II. Osmotic minipumps (model 2001 Alzet, Durect, Cupertino, CA) were implanted into 6- to 8-wk-old LDLR^{-/-} male mice (5 mice/group). Pumps were filled with saline vehicle or solutions of losartan (catalog no. L-158086-005H067, Merck) that delivered 30 mg·kg⁻¹·day⁻¹ for 7 days. Losartan, an AT₁R-specific antagonist (58), was used at a dose that

previously afforded AT₁R blockade (14). For ANG II infusion studies, saline or ANG II (500 ng·kg⁻¹·min⁻¹) was infused by osmotic minipump (model 2004) for 28 days. Pumps were placed in the subcutaneous space of ketamine-xylazine (50 and 5 mg/kg, respectively, ip)-anesthetized mice through a small incision in the flank of the neck, which was closed with surgical glue.

Western blotting analysis of AGT. As described previously (15), a chicken antibody was developed against mouse AGT. The sequence (EEEQPTTSVQPGSPE) used to generate the antibody was from the carboxyl terminus of AGT and did not react with other angiotensin peptides.

Mouse plasma samples were resolved electrophoretically, transferred to polyvinylidene difluoride membranes, and analyzed by Western blot as described elsewhere (37). Polyvinylidene difluoride membranes were incubated with chicken anti-mouse AGT IgY (1:500–1:1,000 dilution in Tris-buffered saline with 5% nonfat dry milk and 0.1% Tween 20) and then with a peroxidase-conjugated rabbit anti-chicken IgY (1:5,000–1:10,000 dilution; Jackson Immuno-Research). The immunoreactivity was visualized with an enhanced chemiluminescence Western blotting detection kit (Pierce, Rockford, IL). Kodak Image (Image Station, 440CF) was used for quantitative assessment of band densities.

Measurement of angiotensin peptides. Angiotensin peptides were resolved by HPLC as described previously (15). HPLC fractions were assayed using an anti-ANG II antibody that exhibited 100% cross-reactivity to ANG III and ANG-(3–8) (ANG IV) and 80% reactivity to ANG-(4–8).

Statistical analysis. Values are means ± SE. Statistical significance between groups was assessed by an unpaired *t*-test (Bonferroni/Dunn)

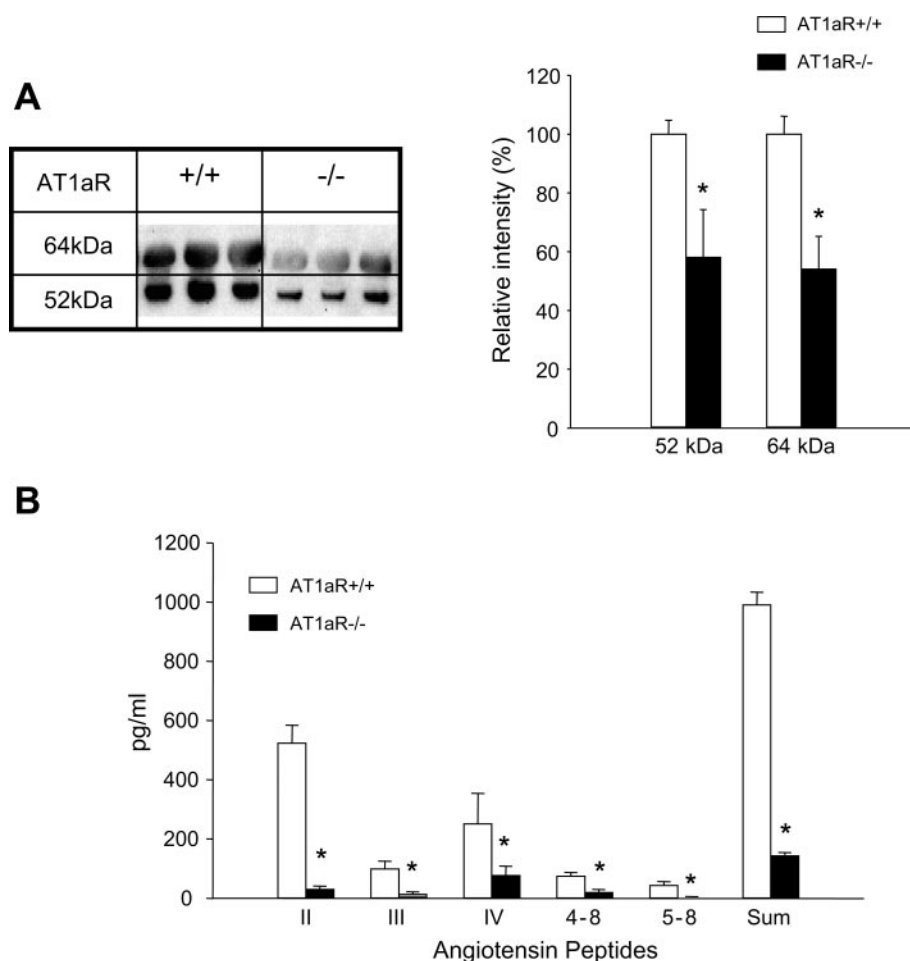


Fig. 3. Effect of AT_{1a}R deficiency on plasma AGT (A) and angiotensin peptide (B) concentrations. Densitometric analysis of plasma AGT protein bands demonstrates a significant decrease in levels of 52- and 64-kDa AGT protein bands in AT_{1a}R^{-/-} mice. Plasma concentrations of angiotensin peptides were significantly decreased in AT_{1a}R^{-/-} mice, resulting in a marked reduction in the sum concentration of plasma angiotensins. Values are means ± SE (*n* = 4–6 mice/group). **P* < 0.05.

or by ANOVA using SigmaStat version 3.0. Data sets were tested to ensure that they complied with the constraints of parametric tests. $P < 0.05$ was considered statistically significant.

RESULTS

Differential expression of components of the RAS in adipose tissue vs. liver. We initially determined AGT, AT_{1a}R, angiotensin type 1b (AT_{1b}) receptor (AT_{1b}R), and AT₂R mRNA abundance in epididymal adipose tissue compared with liver of LDLR^{-/-} mice. Adipose mRNA abundance of AGT was 68% of that in liver (Fig. 1A). In mouse adipose tissue and liver, we detected AT_{1a}R and AT₂R, but not AT_{1b}R mRNA (data not shown). AT_{1a}R mRNA expression was lower (2-fold) in adipose tissue than in liver (Fig. 1B), whereas AT₂R mRNA expression was 57% greater in adipose tissue than in liver (Fig. 1C).

Effects of AT_{1a}R deficiency on adipose tissue, liver, and systemic RAS. To evaluate the effects of endocrine feedback regulation by ANG II on the RAS, we determined the effects of AT_{1a}R deficiency on the mRNA abundance of the AT₂R and AGT in adipose tissue and liver. AT₂R mRNA abundance was not altered in adipose tissue from AT_{1a}R^{-/-} mice (AT₂R-to-18S rRNA ratio = 0.29 ± 0.13 and 0.27 ± 0.11 in AT_{1a}R^{+/+} and AT_{1a}R^{-/-} mice, respectively, $P = 0.929$). Similarly, adipose mRNA abundance of AGT was unaltered in AT_{1a}R^{-/-} mice (Fig. 2A). In contrast, liver AGT mRNA abundance was decreased (by 38%) in AT_{1a}R^{-/-} mice (Fig. 2B). Plasma AGT exhibited two protein bands (52 and 64 kDa), both of which were decreased (2-fold) in AT_{1a}R^{-/-} mice (Fig. 3A). To determine whether differential glycosylation gave rise to the two AGT protein bands (37, 41), we incubated plasma with peptide *N*-glycosidase F, which resulted in a single ~50-kDa protein AGT band (data not shown). Reductions in plasma AGT concentrations were accompanied by a decrease in angiotensin peptide concentrations in plasma of AT_{1a}R^{-/-} mice (Fig. 3B).

Effects of AT₂R deficiency on adipose and systemic RAS. Adipose tissue from AT₂R^{-/-} mice exhibited increased AGT (by 63%; Fig. 4A) and AT_{1a}R (by 83%; Fig. 4B) mRNA abundance. In contrast, liver AGT mRNA abundance was unaltered by AT₂R deficiency (data not shown). Elevations in adipose expression of these RAS components were accompanied by an increase in plasma concentrations of angiotensin peptides (Fig. 4C). To determine whether these elevated angiotensin peptide concentrations contributed to enhanced RAS expression in adipose tissue through the AT_{1a}R, we administered losartan to AT₂R^{+/+} and AT₂R^{-/-} mice. Elevations in the mRNA abundance of AGT and AT_{1a}R in adipose tissue were totally ablated in losartan-infused mice (Fig. 4, A and B). Similar to results obtained in AT_{1a}R^{-/-} mice, losartan administration decreased AGT mRNA abundance (by 53%) in the liver of AT₂R^{-/-} and AT₂R^{+/+} mice.

Adipose AGT and AT_{1a}R mRNA are increased by infusion of ANG II and paralleled by an increase in systemic AGT protein. Results described above suggest that elevated systemic angiotensin peptide concentrations stimulate the adipose RAS. We infused LDLR^{-/-} mice with ANG II at a dose that has been demonstrated to increase blood pressure (32). Infusion of ANG II did not change AT₂R mRNA expression in epididymal adipose tissue (AT₂R-to-18S rRNA ratio = 0.14 ± 0.01 and 0.18 ± 0.02 in saline- and ANG II-infused animals, respec-

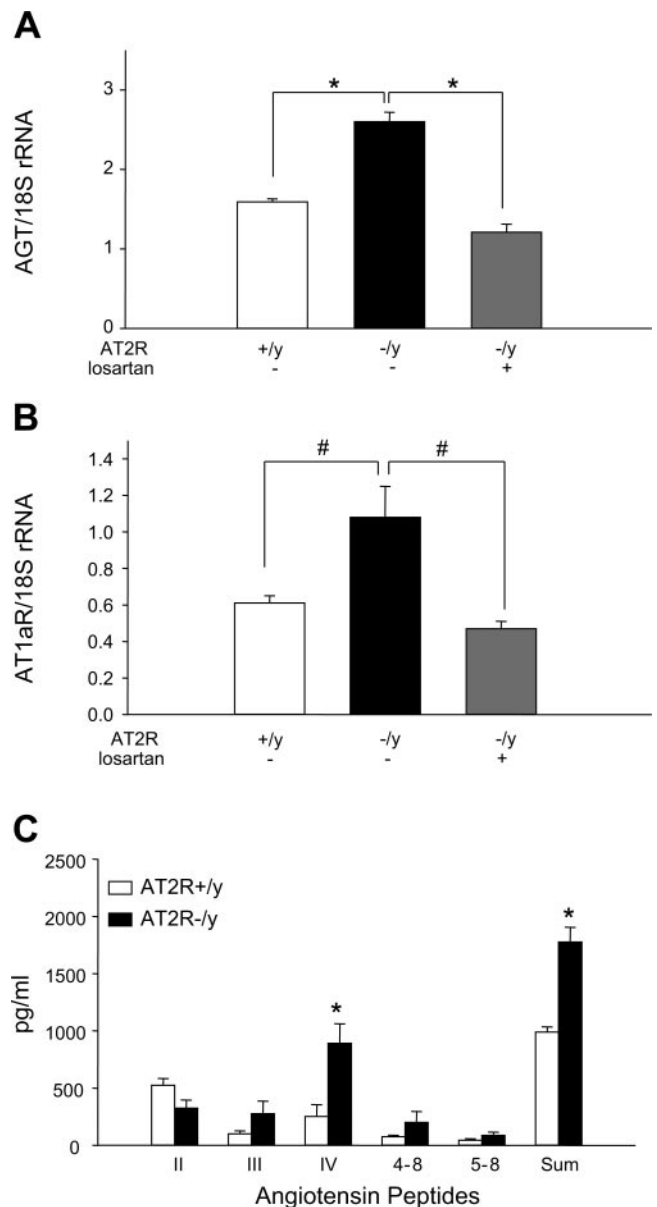


Fig. 4. Effect of AT₂R deficiency on adipose expression of AGT (A), AT_{1a}R (B), and plasma angiotensin peptide (C) concentrations. A and B: AGT and AT_{1a}R mRNA abundance in adipose tissue were increased in AT₂R^{-/-} compared with wild-type mice. Elevations in AT_{1a}R and AGT mRNA abundance were ablated in losartan-treated AT₂R^{-/-} mice. C: angiotensin peptide concentrations in plasma of AT₂R^{+/+} and AT₂R^{-/-} mice. Values are means \pm SE ($n = 4-6$ mice/group). * $P < 0.001$. # $P < 0.01$.

tively, $P = 0.07$). However, infusion of ANG II resulted in a striking increase in the abundance of AT_{1a}R (7.3-fold) and AGT (2.8-fold) mRNA in adipose tissue (Fig. 5). In contrast, there was no effect of ANG II infusion on liver AGT and AT_{1a}R mRNA expression (Fig. 5). Plasma AGT concentrations (64-kDa band) were increased (2-fold) by ANG II infusion (Fig. 6).

DISCUSSION

Our results demonstrate differential endocrine feedback regulation of the RAS in adipose tissue vs. liver. Mechanisms contributing to these effects include differences in AT_{1a}R vs.

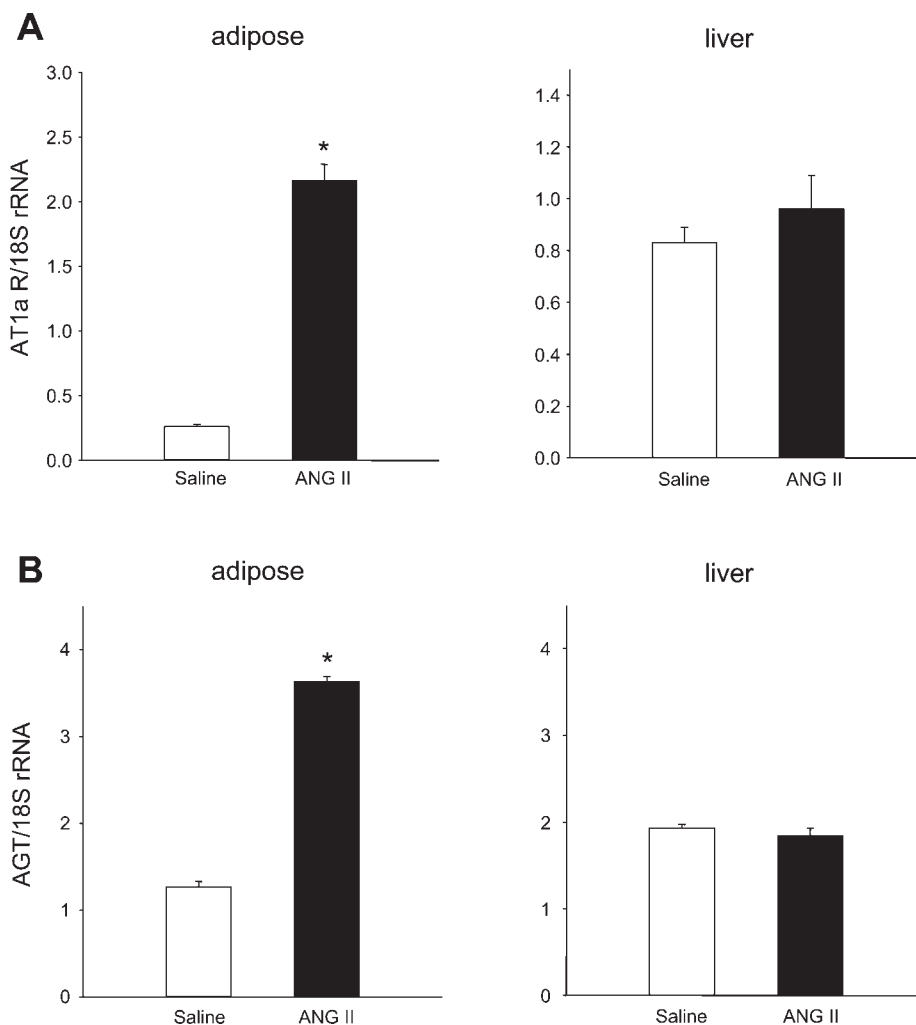


Fig. 5. Effect of chronic infusion of ANG II on $AT_{1a}R$ (A) and AGT (B) mRNA abundances in adipose tissue and liver. Saline or ANG II ($500 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was infused by osmotic minipump for 28 days. $AT_{1a}R$ and AGT mRNA abundances were significantly increased in adipose tissue from ANG II-infused mice. $AT_{1a}R$ and AGT mRNA abundances were unaltered in livers of ANG II-infused mice. Values are means \pm SE ($n = 4\text{--}5$ mice/group). * $P < 0.001$.

AT_{2R} expression between adipose tissue and liver and differences in the ability of systemic ANG II to regulate $AT_{1a}R$ expression in adipose tissue compared with liver. In $AT_{1a}R^{-/-}$ mice, we found that AGT protein concentrations in plasma parallel the mRNA abundance in liver. This suggests that $AT_{1a}R$ -mediated regulation of AGT in mouse liver serves a primary role in determining the circulating AGT and ANG II concentrations. Results from $AT_{2R}^{-/-}$ mice demonstrate that a primary mediator of differential regulation of AGT by ANG II in liver vs. adipose tissue relates to expression of the AT_{2R} in adipose tissue. In $AT_{2R}^{-/-}$ mice, adipose expression of the $AT_{1a}R$ and AGT paralleled plasma angiotensin peptide concentrations. Interestingly, in states of elevated systemic ANG II, marked elevations in $AT_{1a}R$ expression in adipose tissue resulted in pronounced regulation of AGT in adipose tissue, but not in liver. These results suggest that adipose tissue contributes to the concentration of plasma AGT and/or ANG II under conditions of high systemic ANG II.

Tissue-specific regulation of the $AT_{1a}R$ by ANG II. Previous studies have reported elevations in $AT_{1a}R$ mRNA expression in a tissue-specific manner in states of elevated systemic ANG II, such as low salt, chronic ANG II infusion, and renovascular hypertension (17, 18, 29, 45, 53). However, regulation of $AT_{1a}R$ expression by ANG II has not been examined in

adipose tissue. Our results extend previous findings by demonstrating a robust effect of ANG II to increase $AT_{1a}R$ mRNA abundance in adipose tissue. In this study, a physiological consequence of ANG II regulation of $AT_{1a}R$ expression in adipose tissue was the promotion of AGT expression. However, ANG II has been demonstrated to exert a variety of AT_{1R} -mediated effects at adipocytes (8, 57, 59, 60). Thus upregulation of $AT_{1a}R$ mRNA in adipose tissue in states associated with high plasma ANG II concentrations could result in changes in a variety of adipocyte functions. In contrast to findings in adipose tissue, our results do not support regulation of liver $AT_{1a}R$ expression after chronic ANG II infusion. These results are in agreement with previous data demonstrating no change in liver $AT_{1a}R$ gene expression with ANG II infusion (29). The relatively high mRNA abundance of the $AT_{1a}R$ in liver vs. adipose tissue may have contributed to differences in the ability of ANG II to upregulate $AT_{1a}R$ expression between these tissues.

No studies have examined differential expression of upstream transcriptional binding partners in adipose tissue vs. liver. The AT_{1a} promoter exhibits a high degree of complexity in its regulation, and certain polymorphisms are thought to be associated with differential regulation of this receptor (12). The transcription factor Sp1 associates with a $-98/-79$ and

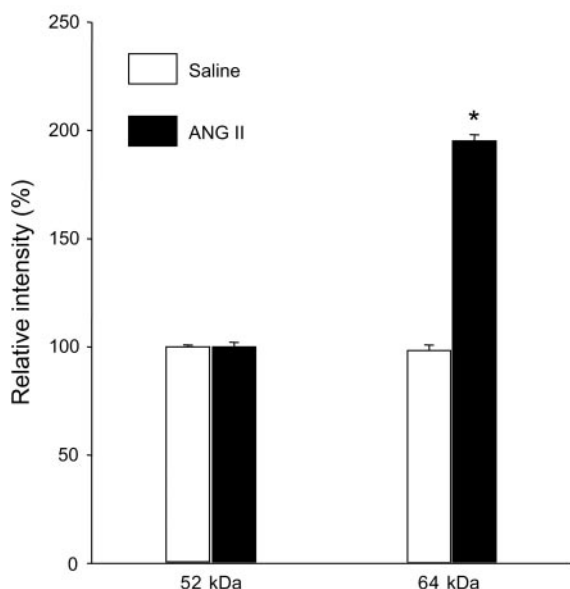
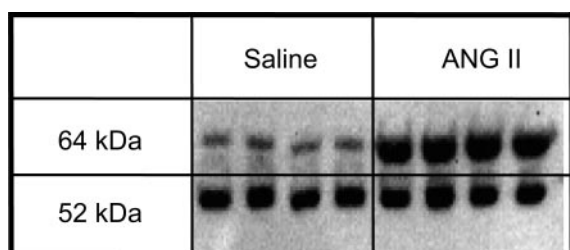


Fig. 6. Effect of chronic infusion of ANG II on plasma AGT. Saline or ANG II ($500 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was infused by osmotic minipump for 28 days. The 64-kDa protein band of immunoreactive AGT was markedly increased by ANG II infusion. Values are means \pm SE ($n = 4$ mice/group). * $P < 0.001$.

–58/–34 region sequence (GC-rich) of the AT_{1a}R promoter in A10 smooth muscle cells and a human trophoblast cell line (19, 36). Expression of the AT_{1a}R is induced by ANG II in vascular smooth muscle cells but is inhibited in the adrenal gland (12). However, no studies have specifically examined whether liver and/or adipose tissue AT_{1a}R is associated with regulation by Sp1. Furthermore, a negative *cis*-regulatory element between –489 and –331 on the AT_{1a}R promoter was identified in PC-12 cells and rat brain, but not in liver (46). These results suggest possible tissue-specific regulation of the AT_{1a}R via different transcription factors. Alternatively, cross talk between the AT_{1a}R and the AT_{2}R in adipose tissue, but not in liver, may have influenced differential ANG II regulation of AT_{1a}R expression between these tissues.

Tissue-specific regulation of AGT by ANG II. Adipose tissue has been demonstrated to express the majority of components required for the synthesis of ANG II (7, 22). Of these, AGT has been consistently demonstrated at a high level of expression in mouse (23, 35, 39, 50), rat (3, 27), and human (25, 26, 61) adipocytes. Previous investigators have demonstrated a variety of factors that regulate adipose AGT expression (1–3, 6, 10, 23, 24, 31, 35, 39, 49, 54, 55). In contrast, relatively few studies have examined endocrine feedback regulation of adipose-derived AGT by angiotensin peptides. ANG II was reported to decrease AGT expression in differentiating human preadipo-

cytes (51) but had no effect when incubated with primary mammary adipocytes (28). Similar to responses observed in primary mammary adipocytes, we did not observe alterations in AGT mRNA expression in adipose tissue of $\text{AT}_{1a}\text{R}^{-/-}$ mice. In contrast, our results demonstrate robust regulation of AGT expression in adipose tissue of $\text{AT}_{2}\text{R}^{-/-}$ mice. It is conceivable that the AT_{2}R , by providing a competing site for ANG II binding in adipocytes, may have reduced the availability of ANG II to occupy the AT_{1a}R and, subsequently, regulate AGT expression. In support of this conclusion, the infusion of ANG II, which would elevate systemic ANG II concentrations at adipocytes and afford greater AT_{1a}R occupation, resulted in an upregulation of AT_{1a}R and AGT mRNA expression in adipocytes.

Taken together, our results demonstrate that in the presence of low or normal concentrations of plasma ANG II, the AT_{1a}R is not a primary mediator of AGT regulation in adipose tissue. In contrast, the AT_{1a}R is a primary regulator of AGT by ANG II in liver under these circumstances. In contrast, when AT_{2}R expression is reduced in adipose tissue or in a situation of elevated systemic ANG II, our results support a primary role for AT_{1a}R s in the regulation of adipose AGT expression.

Relationship between adipose and liver AGT regulation and systemic ANG II. Under most physiological conditions, ANG II generation is largely dependent on ANG I cleavage by renin, the rate-limiting enzyme in the generation of systemic ANG II. However, in most species, the Michaelis-Menten constant for renin approximates the plasma AGT concentration, suggesting that alterations in circulating AGT can influence ANG II synthesis (20). In mice, several lines of evidence suggest that AGT may limit the rate of ANG II synthesis. Graded doses of sheep AGT resulted in a dose-dependent increase in blood pressure in mice, but not in rats, suggesting that AGT was limiting in mouse plasma (40). Similarly, a dose-dependent increase in blood pressure was observed in mice treated with increasing doses of intravenously infused AGT, coincident with an elevation in systemic ANG II concentrations (11). Collectively, these results suggest that regulation of AGT may serve as a primary mechanism for control of angiotensin peptide concentrations in mice.

Our findings demonstrate that liver AGT mRNA expression paralleled angiotensin peptide concentrations in $\text{AT}_{1a}\text{R}^{-/-}$ mice, supporting an important role of liver-derived AGT in the production of systemic ANG II. Interestingly, in $\text{AT}_{2}\text{R}^{-/-}$ mice, the plasma concentrations of angiotensin peptides began to parallel AGT expression in adipose tissue, but not in liver. A primary mechanism for this effect was a striking increase in AT_{1a}R mRNA expression in adipose tissue of $\text{AT}_{2}\text{R}^{-/-}$ mice. The ability of adipose tissue to contribute to AGT in plasma has been suggested from studies in transgenic mice overexpressing AGT in adipose tissue. These mice exhibit elevations in circulating concentrations of AGT and blood pressure (43). Results from our studies extend these findings by demonstrating that marked upregulation of AT_{1a}R mRNA expression by ANG II makes adipose tissue a more prominent contributor to circulating AGT concentrations and, thereby, ANG II concentrations. In addition, these results demonstrate that ANG II can modulate adipose-derived AGT in a positive endocrine feedback loop through effects at the AT_{1a}R . Thus, under conditions of elevated systemic ANG II, our results would suggest that adipose tissue becomes a prominent source of AGT. We

previously reported that diet-induced obesity in rats was associated with increased adipose AGT mRNA expression that correlated positively to plasma angiotensin peptide concentrations and blood pressure (3). Interestingly, liver AGT expression was not altered in obese rats, suggesting that adipose tissue contributed significantly to systemic AGT/angiotensin concentrations. Recent studies suggest that reductions in body weight in obese patients were associated with decreased plasma AGT concentration, which correlated to waist circumference (21). The positive-feedback endocrine regulation of adipose-derived AGT by ANG II demonstrated in this study may contribute to overactivity of the RAS in obesity-associated disorders.

In conclusion, results from this study demonstrate that adipose tissue differs from liver in the regulation of AGT by ANG II because of differences in the AT_{1a}R/AT₂R populations of these tissues. Expression of the AT₂R in mouse adipose tissue masks the effects of ANG II to increase AGT expression through the AT_{1a}R. When AT₂Rs are ablated, plasma concentrations of ANG II begin to parallel AGT expression in adipose tissue, and not in liver. In addition, chronic infusion of ANG II resulted in marked upregulation of AT_{1a}R and AGT mRNA expression in adipose tissue, but not in liver. In ANG II-infused mice, plasma AGT concentrations paralleled adipose AGT mRNA expression. These results demonstrate that ANG II regulates adipose AGT via activation of the AT_{1a}R and that this positive-feedback loop may play an important role in the pathogenesis of ANG II-related cardiovascular diseases.

GRANTS

These studies were supported by National Heart, Lung, and Blood Institute Grants HL-73085 (L. A. Cassis) and HL-62846 (A. Daugherty).

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