Regulation of Angiotensin II Type 1 Receptor mRNA and Protein in Angiotensin II–Induced Hypertension

Lisa M. Harrison-Bernard, Samir S. El-Dahr, Denise F. O’Leary, L. Gabriel Navar

Abstract—Chronic elevations of circulating angiotensin II (Ang II) cause sustained hypertension and enhanced accumulation of intrarenal Ang II by an AT₁ receptor–dependent process. The present study tested the hypothesis that chronic elevations in circulating Ang II regulate AT₁ mRNA and protein expression in a tissue-specific manner. Sprague-Dawley rats were infused with Ang II (80 ng/min) or vehicle subcutaneously for 13 days via osmotic minipump. On day 12, systolic blood pressure averaged 186±2 mm Hg in Ang II–infused rats compared with rats given vehicle (121±2 mm Hg). Plasma renin activity was markedly suppressed in the Ang II–infused rats compared with vehicle-infused rats (0.1±0.01 versus 4.9±0.9 ng of Ang I · mL⁻¹ · h⁻¹; P<0.05). Semiquantitative reverse transcription polymerase chain reaction using rat AT₁A and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH)–specific primers was followed by Southern blot hybridization using specific radiolabeled cDNA or oligonucleotide probes. The results showed that the ratios of AT₁A/GAPDH mRNA in the kidney (0.19±0.05 versus 0.26±0.03) and liver (2.8±0.9 versus 3.0±0.5) were comparable in Ang II– and vehicle-infused rats. In contrast, AT₁A/GAPDH mRNA levels were increased in the adrenal glands of Ang II–infused rats (0.49±0.04 versus 0.36±0.02; P<0.05). Western blot analysis showed that AT₁ protein levels in the kidney and liver were also similar in the two groups. Therefore, these results indicate that renal and liver AT₁ receptor gene expression is maintained in Ang II–induced hypertension. The failure to downregulate AT₁ receptor mRNA and protein levels thus allows the sustained effects of chronic elevations in Ang II to elicit progressive increases in arterial pressure. (Hypertension. 1999;33[part II]:340-346.)

Key Words: kidney l liver l adrenal gland l gene expression l antibodies

Chronic administration of angiotensin II (Ang II) is a useful experimental model of Ang II–dependent hypertension that mimics 2-kidney, 1 clip (2K1C) Goldblatt hypertension. The mechanisms responsible for the progressive nature of Ang II–induced hypertension are multifarious and incompletely understood. We previously demonstrated that chronic infusion of initially a subpressor dose of Ang II into uninephrectomized rats produces a slowly developing hypertension that is associated with diminished renal renin content and mRNA, enhanced renal ACE activity, maintained renal and hepatic angiotensinogen mRNA levels, and an AT₁–mediated augmentation of intrarenal Ang II content.¹⁻⁴ However, the contribution of the expression of the AT₁ receptor to the hypertensinogenic effect of Ang II has not been established. Because most of the hypertensinogenic actions of the renin-angiotensin system are mediated by the binding of Ang II to the AT₁ receptor, the predominant receptor subtype in adult animals, and the availability of AT₁ receptors essentially determines the efficacy of the renin-angiotensin system, we studied the tissue-specific expression of the AT₁ receptor after chronic low-dose infusions of Ang II in the rat. In particular, the regulation of AT₁ receptor mRNA and protein expression was determined in the kidney, liver, and adrenal gland.

Methods

Animals and Tissue Preparation
Male Sprague-Dawley rats (Charles River Labs, Wilmington, MA) were housed in wire cages and maintained in a temperature-controlled room regulated on a 12-hour light/dark cycle. Rats had free access to water and standard rat chow (Ralston-Purina). The experimental protocol was approved by the Tulane University Animal Care and Use Committee. Rats (230±4 g body wt; n=17) were anesthetized with sodium pentobarbital (50 mg/kg IP), and an osmotic minipump (model 2002; Alza Corp) was implanted subcutaneously at the dorsum of the neck. Rats were selected at random to receive Ang II infusion (n=8; Novabiochem) at a rate of 80 ng/min (350 ng · kg⁻¹ · min⁻¹) or vehicle (n=9; 5% acetic acid) for a period of 13 days. Systolic blood pressures were measured in conscious rats 1 day before surgery and on days 6 and 12 of the infusion using tail-cuff plethysmography (model 52-0338; Harvard Apparatus). Blood and tissue samples (kidneys, liver, adrenal glands) were harvested on day 13 after rapid decapitation. Trunk blood was collected into chilled tubes containing EDTA. Plasma was separated and stored at −20°C until assayed for plasma renin activity using a commercially available Ang I RIA kit (Incstar) as described previously.¹ Liver and kidneys were removed for protein and RNA extraction. Adrenal glands were removed for RNA extraction. Samples were snap-frozen in liquid nitrogen and stored at −80°C until processed.

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**AT1A Receptor mRNA Analyses**

Total RNA was extracted using the RNeasy Midi Kit (Qiagen) according to the manufacturer’s specifications. The extracted RNA was quantified spectrophotometrically by absorbance at 260 nm, dissolved in sterile water, and stored at −80°C until use. Integrity of RNA was documented by agarose gel electrophoresis and observation of 28S and 18S ribosomal RNA.

**RT-PCR**

cDNA was synthesized by use of SuperScript II RT (GIBCO BRL). The reaction mixture contained DNase I–digested RNA (kidney, 5 μg; liver, 3 μg; adrenal, 2.5 μg). 10× PCR buffer (200 mmol/L Tris·HCl, pH 8.4, 500 mmol/L KCl, 10 mmol/L MgCl2), 100 ng random hexamers, 10 mmol/L concentration each of deoxynucleoside triphosphate, 0.1 mmol/L DTT, and 200 U RT for a total volume of 23 μL. RT was omitted in control samples. Positive control for the RT step consisted of control RNA provided by the manufacturer. The reaction mixture was incubated at 25°C for 10 minutes and 42°C for 50 minutes, heated to 70°C for 15 minutes, and chilled on ice. RNase H (2 U) was added to each tube and incubated at 37°C for 20 minutes. RT mixture was then diluted with 10× PCR buffer, followed by the addition of upstream and downstream primers and 2.5 U Taq DNA polymerase (GIBCO BRL) for a final volume of 50 μL. The reaction was initiated by heat denaturation of RNA-cDNA hybrid at 95°C for 1 minute, annealing of the primers (50 pmol for AT1A, 25 pmol glyceraldehyde-3-phosphate-dehydrogenase [GAPDH]), and extension at 72°C. The cycle was repeated 25 times using a programmable PCR thermocycler (Perkin-Elmer Cetus Instruments). Details of each PCR are described. PCRs for GAPDH and AT1A were performed from the same RT products to allow the data to be expressed as a ratio to GAPDH. The appropriate PCR reaction cycle number was determined for the two targeted mRNAs by including 0.5 μCi [α-32P]dCTP in the PCR and performing 15 to 35 cycles of amplification. PCR products were run on 2% agarose gel, and the bands were excised, dried at 37°C overnight, and stained with 0.1% Coomassie blue R250 to visualize the protein band exhibiting linearity of the hybridization signals with values of 6 r2. Signals were detected by autoradiography and quantified by scanning laser densitometry (Ultrascan; Pharmacia LKB).

**DNA Probe Radiolabeling**

Probes were labeled with 50 μCi [α-32P]dCTP using random primers DNA labeling system (GIBCO BRL) and purified on Sephadex G-50 Nick columns (Pharmacia). The cDNA probe used was a human GAPDH cDNA (Clontech). Five pmol of the oligonucleotide (lower AT1A or GAPDH primer) was 5' end-labeled with 50 to 70 μCi [γ-32P]ATP using T4 polynucleotide kinase (20 U; GIBCO BRL) and purified on a Sephadex G25 column (Pharmacia).

**Slot Blot Analysis**

Slot blots were prepared by serial 2-fold dilutions (4 to 1 μg) of total RNA from individual animals as described previously. Blots were hybridized to the AT1A oligo probe and rehybridized with a GAPDH oligo probe to account for loading variations (2 to 3 months allowed for 32P decay). Signals were detected by autoradiography and quantified by scanning laser densitometry (Ultrascan; Pharmacia LKB).

**AT1 Receptor Protein Analysis**

Proteins were extracted from kidney and liver samples after homogenization as described previously and assayed by the method of Lowry et al.

**Western Blot Analysis of AT1 Receptor**

Kidney (50 μg) or liver (25 μg) samples were electrophoretically separated by 3% to 10% stacking Tris-glycine gel at 100 V for 2 hours (10% SDS, 24 mmol/L Tris base, 192 mmol/L glycine) and transferred (20% methanol, 12 mmol/L Tris base, 96 mmol/L glycine) to nitrocellulose membrane (0.45; BioRad) for 90 minutes at 25 V according to the manufacturer’s specifications (XCell II Mini-Cell; Novex). Molecular weight markers (10- to 250-kDa rainbow; Amersham; and 10-kDa protein ladder, GIBCO BRL) were used to determine approximate molecular mass. Western blot analysis was performed as described previously with minor modifications. Blots were incubated with the primary antibody (1) anti-peptide (8 to 17 amino acids, AT1 monoclonal antibody 6313/G2, 1:50; GP Vinson) or (2) anti-peptide AT1 polyclonal antibody 1:200 (15 to 24 amino acids, SC-1173; Santa Cruz) for 3 hours, washed, incubated with the secondary antibody conjugated to horseradish peroxidase (1:1500) for 1 hour, and washed. Detection was accomplished using enhanced chemiluminescence Western blotting (ECL; Amersham), and the blots were exposed to x-ray film (Hyperfilm-ECL; Amersham). For preadsorption studies, the polyclonal antibody and the synthetic peptide antigen (20 μg; SC-1173P) were incubated overnight at 4°C. Kidney (50 μg) and liver (25 μg) samples were separated in duplicate on each of 2 gels. One gel was stained with 0.1% Coomassie blue R250 to visualize the protein bands for total protein quantification. The proteins from the second gel were electrophoretically transferred to nitrocellulose and incubated with the primary polyclonal antibody or preadsorbed antibody as described above.

**Statistical Analysis**

Results are expressed as mean±SEM. For slot blots, regression lines were calculated for each tissue sample from the ratio of the densitometric values of the 3 serial dilutions, and only those samples exhibiting linearity of the hybridization signals with values of r≥0.9 were accepted for further consideration. For Southern and slot blot analyses, AT1A signals were factored for GAPDH. The data were analyzed using unpaired t test or Mann-Whitney rank sum test. Statistical significance is defined at a value of P≤0.05.
Results

Body Weight, Blood Pressure, and Plasma Renin Activity

Body weights in the two groups at the initiation of the study were similar (Ang II, 226±4 g, n=8; vehicle, 225±4 g, n=9). On day 13 of the infusion, average body weight in the Ang II–infused animals was significantly reduced compared with vehicle (292±7 versus 343±9 g). Systolic blood pressures (Figure 1) were identical in the 2 groups before implantation of the osmotic minipumps (121±2 mm Hg). On days 6 and 12 of infusion, pressures were significantly elevated to 168±3 and 186±12 mm Hg in the Ang II–infused rats compared with vehicle-infused rats (118±3 and 119±3 mm Hg). Plasma renin activity was markedly suppressed in the Ang II–infused rats compared with vehicle-infused rats (0.1±0.01 versus 4.9±0.9 ng of Ang I·mL⁻¹·h⁻¹, n=7 and 9).

AT₁ Receptor Antibody Specificity

We documented the specificity of the anti-peptide polyclonal AT₁ receptor antibody by performing Western blot analysis of liver and kidney protein using the antibody preadsorbed with the synthetic peptide antigen. Figure 2 illustrates a Western blot autoradiograph comparing liver and kidney AT₁ receptor protein detected using the primary antibody shown on the left and preadsorbed antibody on the right. Densitometric analysis of the bands indicates that the intensity of the signal was >90% decreased using the preadsorbed antibody. Equal loading was determined by Coomassie blue staining of the duplicate gel. The results demonstrate that the polyclonal AT₁ receptor antibody is specific for the ≈46-kDa AT₁ receptor protein in these tissues. We previously reported that adsorption of the monoclonal AT₁ antibody by the antigenic peptide eliminates renal vascular and tubular AT₁ receptor antibody immunostaining, demonstrating that the monoclonal antibody is specific for the AT₁ receptor protein. The antigenic peptide sequences for the two antibodies correspond to portions of the AT₁ receptor protein that are identical for the AT₁A and AT₁B receptor subtypes.

Kidney AT₁ Receptor mRNA and Protein Expression

Western blot analysis of AT₁ receptor protein from whole kidney homogenates using the monoclonal antibody in Ang II–infused animals showed that the receptor protein levels remain unchanged during chronic Ang II–induced hypertension (42±6 versus 36±3 du; n=5) (Figure 3A). Similar results were obtained using the polyclonal antibody for Ang II–infused (9±3 du; n=4) and vehicle-infused (6±1 du; n=5) rats. Renal AT₁A mRNA expression was assessed using semiquantitative RT-PCR and Southern blot analysis (Figure 3B). Renal AT₁A mRNA levels were not significantly different between the vehicle- and Ang II–infused animals. The ratio of renal AT₁A to GAPDH densitometric signals was 0.66±0.07 in vehicle-infused (n=9) and 0.47±0.10 in Ang II–infused (n=8) animals. In addition, semiquantification of kidney AT₁A mRNA by incorporation of [α-³²P]dCTP confirmed the results obtained by Southern blot analysis. The ratio of AT₁A and GAPDH PCR products (cpm) was 1.1±0.4 (n=4) in vehicle-infused and 1.1±0.3 (n=4) in Ang II–infused rats. AT₁A mRNA slot blot analysis of 4, 2, and 1 µg of total kidney RNA is shown in Figure 3C and demonstrates that AT₁A mRNA levels were not different between the 2 groups, in agreement with the RT-PCR analysis. The ratios of kidney AT₁A and GAPDH densitometric signals were 4.2±0.4 versus 4.5±0.3, 3.2±0.2 versus 3.3±0.3, and 3.6±0.5 versus 3.6±0.3 for vehicle-infused (n=9) versus Ang II–infused (n=7) animals at 4, 2, and 1 µg RNA, respectively.

Liver AT₁ Receptor mRNA and Protein Expression

Western blot analysis of liver AT₁ receptor protein from Ang II– and vehicle-infused animals showed that the receptor protein levels were not altered during Ang II–induced hypertension (52±3 versus 52±3 du, polyclonal primary antibody; 11±2 versus 10±2 du, monoclonal: Figure 4A). Semiquantification of liver AT₁A mRNA by RT-PCR demonstrated that the receptor gene expression is not altered by chronic Ang II infusion (Figure 4B). The ratio of liver AT₁A to GAPDH Southern blot densitometric signals was 3.0±0.5 in vehicle-infused (n=9) and 2.8±0.9 in Ang II–infused (n=8) animals. Semiquantification of liver AT₁A mRNA by incorporation of [α-³²P]dCTP confirmed the results obtained by Southern blot analysis. The ratio of AT₁A and GAPDH PCR products (cpm)
was 0.42±0.09 (n=5) in vehicle-infused and 0.30±0.03 (n=4) in Ang II-infused animals (P=0.14). Slot blot analysis of liver AT1A mRNA in vehicle- and Ang II–infused animals is shown in Figure 4C. Liver AT1A gene expression was not altered during Ang II–induced hypertension. The ratios of liver AT1A and GAPDH densitometric signals were 2.9±0.3 versus 3.1±0.1, 3.0±0.2 versus 3.2±0.3, and 3.8±0.7 versus 4.1±0.5 for vehicle-infused (n=9) versus Ang II–infused (n=8) animals at 4, 2, and 1 μg RNA, respectively.

### Adrenal AT1A Receptor mRNA Expression

Adrenal AT1A mRNA was analyzed as described above. RT-PCR and Southern blot analysis demonstrated a significant elevation in AT1A mRNA in the adrenal gland of Ang II–infused animals (Figure 5A). The ratios of adrenal AT1A to GAPDH Southern blot densitometric signals were 0.36±0.02 in vehicle-infused (n=8) and 0.49±0.04 in Ang II–infused (n=8) animals (P<0.01). By slot blot analysis, AT1A/GAPDH mRNA tended to be higher but did not reach statistical significance (Figure 5B). The ratios of adrenal AT1A and GAPDH densitometric signals were 0.58±0.05 versus 0.68±0.08, 0.75±0.05 versus 0.87±0.08, and 0.98±0.12 versus 0.87±0.08 for vehicle-infused (n=8) versus Ang II–infused (n=6) animals at 4, 2, and 1 μg RNA, respectively.

### Discussion

In agreement with previous studies, chronic low-dose infusion of Ang II produced significant elevations in systolic blood pressure and reductions in plasma renin activity. After 13 days of infusion, we found that kidney and liver AT1A mRNA and protein levels were maintained under conditions of chronic elevations in Ang II. However, in the adrenal gland, AT1A mRNA expression was significantly elevated. These results indicate that renal AT1 receptor gene expression is not suppressed in Ang II–induced hypertension. The failure to downregulate AT1 receptor mRNA and protein levels thus allows the sustained effects of chronic elevations in Ang II to elicit progressive increases in arterial pressure.

The AT1 receptor is 1 of 2 major Ang II receptor subtypes, which has been characterized pharmacologically and cloned. In rodents, 2 subtypes of AT1 receptors exist (AT1A, AT1B). The AT1A and AT1B receptor subtypes are 95% homologous at the amino acid level, but their tissue distributions and chromosomal locations are different. In the rat kidney, AT1A mRNA expression has been reported to be 3- to 8-fold higher than AT1B mRNA levels. Using RT-PCR techniques, Bouby et al reported that AT1A mRNA was the predominant subtype in all microdissected nephron segments, with the exception of the glomerulus, where AT1B mRNA levels were higher than AT1A mRNA levels. In agreement with the AT1 mRNA localization, we have previously shown by immunohistochemical analysis that the AT1 receptor protein is present in all nephron segments. The liver is almost exclusively of the AT1A subtype. Therefore, the AT1A receptor subtype is involved in the majority of the hepatic and renal effects of Ang II. However, the adrenal gland expresses equal or 2-fold higher AT1B mRNA levels compared with AT1A mRNA. In the present study, the

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**Figure 3.** Kidney AT1 receptor mRNA and protein expression. A, Top, Western blot of renal AT1 receptor protein levels in Ang II– and vehicle-infused rats detected using a monoclonal antibody (lanes 1 through 5, vehicle infused; lanes 6 through 10, Ang II infused). Bottom, Similar renal AT1 receptor protein density in vehicle-infused (hatched bar; n=5) and Ang II–infused (solid bar; n=5) rats. B, Top, Representative Southern blot showing AT1A and corresponding GAPDH mRNA levels (lanes 1, 3, and 5, vehicle infused; lanes 2, 4, and 6, Ang II infused). Bottom, Ratio of the densitometric analysis of the autoradiographic signals for AT1A and GAPDH in vehicle-infused (hatched bar; n=9) and Ang II–infused (solid bar; n=8) rats. C, Top, representative autoradiograph of AT1A mRNA slot blot hybridized with radiolabeled AT1A-specific oligonucleotide. Membranes were reprobed with radiolabeled GAPDH oligonucleotide probe (lanes 1, 3, and 5, vehicle infused; lanes 2, 4, and 6, Ang II infused). Bottom, Average data for AT1A mRNA detected by slot blot for vehicle-infused (● n=9) and Ang II–infused (○ n=7) rats.
monoclonal and polyclonal antibodies used for Western blot analysis detect both the AT\textsubscript{1A} and AT\textsubscript{1B} receptors. The peptide sequences used to generate the antibodies were from the amino-terminal extracellular tail of the protein in which both the AT\textsubscript{1A} and AT\textsubscript{1B} amino acids sequences are identical. The results of the Western blot analysis using these 2 different AT\textsubscript{1} antibodies indicated that kidney and liver AT\textsubscript{1} protein levels were not significantly altered by chronic Ang II infusion. To determine the regulation of the predominant AT\textsubscript{1} receptor–subtype mRNA expression, RT-PCR and slot blot analyses were performed using an AT\textsubscript{1A}-specific oligonucleotide. The nucleotide sequences chosen were from the 3’ untranslated region of the AT\textsubscript{1A} cDNA.\textsuperscript{5} Interestingly, the 5’ and 3’ flanking sequences of the AT\textsubscript{1B} cDNA are only 35% identical with the AT\textsubscript{1A} cDNA.\textsuperscript{17} Therefore, this study permitted us to assess AT\textsubscript{1} receptor expression at both the protein and mRNA levels to determine the contribution of regulation of the AT\textsubscript{1} receptor at the level of gene transcription as well as at the level of protein expression.

In vitro studies have demonstrated that AT\textsubscript{1} mRNA levels are altered in response to Ang II. Decreases in AT\textsubscript{1} mRNA have been described in aortic vascular smooth muscle cells\textsuperscript{18} and cultured rat glomerular mesangial cells,\textsuperscript{19} whereas increases were reported in cultured rabbit proximal tubule cells.\textsuperscript{20} These data suggest that the regulation of AT\textsubscript{1} mRNA by vascular and tubular structures within the kidney may be differentially regulated and demonstrate that the regulation of the receptor by its ligand may be cell or tissue specific, or both.

Angiotensin-dependent hypertensive animal models have also been used to address the regulation of the AT\textsubscript{1} receptor by alterations in the components of the renin-angiotensin system. In 2K1C Goldblatt hypertensive animals, AT\textsubscript{1A} receptor mRNA levels were not changed in the contralateral intact kidneys but were decreased in the clipped kidneys 2 days\textsuperscript{21} and 1 week after clipping.\textsuperscript{22} AT\textsubscript{1A}, but not AT\textsubscript{1B}, mRNA levels were decreased in both kidneys 4 weeks after clipping in 2K1C rats.\textsuperscript{15} Decreases in vascular and glomerular AT\textsubscript{1} receptor density have been reported in both the clipped and nonclipped kidney of 2K1C rats.\textsuperscript{23} In contrast, AT\textsubscript{1} receptor mRNA was elevated in both the clipped and non-

**Figure 4.** Liver AT\textsubscript{1} receptor mRNA and protein expression. A. Top, Western blot of liver AT\textsubscript{1} receptor protein in Ang II– and vehicle-infused rats detected using polyclonal (top) and monoclonal (bottom) antibodies. Blots are from separate gels run using protein from the same animals in each group (lanes 1, 3, 5, 7, and 9, vehicle infused; lanes 2, 4, 6, 8, and 10, Ang II infused). Bottom, Comparable liver AT\textsubscript{1} receptor protein expression in vehicle-infused (hatched bar) and Ang II-infused (solid bar) rats (n=5) for the polyclonal (left) and monoclonal (right; du >5) primary antibodies. B. Top, Representative blots showing AT\textsubscript{1A} and corresponding GAPDH mRNA levels (lanes 1, 3, and 5, vehicle infused; lanes 2, 4, and 6, Ang II infused). Bottom, Ratio of the densitometric analysis of the autoradiographic signals for AT\textsubscript{1A} and GAPDH in vehicle-infused (hatched bar; n=9) and Ang II-infused (solid bar; n=8) rats. C. Top, Representative autoradiograph of AT\textsubscript{1A} mRNA slot blot hybridized with radiolabeled AT\textsubscript{1A}-specific oligonucleotide in vehicle- and Ang II-infused rats (lanes 1, 3, and 5, vehicle infused; lanes 2, 4, and 6, Ang II infused). Bottom, Graph plotting the average data for AT\textsubscript{1A} mRNA detected by slot blot for vehicle-infused (○; n=9) and Ang II-infused (●; n=8) rats.
clipped kidneys after 10 weeks of 2K1C. The renal expression of the AT₁ receptor during the development of 2K1C hypertension may exhibit a temporal profile. Elevated expression of the renal AT₁ receptor during the latter stages of 2K1C hypertension may explain the maintenance of an elevated blood pressure at a time when plasma renin activity is no longer elevated. In the present study, after 2 weeks of elevations in Ang II, overall kidney AT₁ receptor mRNA and protein levels were maintained at levels similar to vehicle-infused animals, suggesting that the continued synthesis of the AT₁ receptor enabled the sustained effects of circulating Ang II on arterial blood pressure.

Previous studies have evaluated the effects of chronic infusions of Ang II on renal AT₁ receptor expression. Wang et al. reported that 2 weeks of Ang II infusion at doses that do not elevate blood pressure decreases both AT₁A and AT₁B mRNA levels and AT₁ receptor density in the rat kidney. However, Sechi et al. showed that although renal AT₁ mRNA levels were similar in rats infused with Ang II for 1 week compared with vehicle-infused rats, Ang II binding was slightly but significantly decreased by ≈15%. Ang II infusion has been shown to increase and decrease the affinity and number of glomerular Ang II–binding sites. Amiri and Garcia demonstrated that 7-day infusions of nonpressor or pressor doses of Ang II did not alter pregglomerular or glomerular Ang II receptor density. Therefore, there is continued uncertainty regarding the effects of chronic infusions of Ang II on renal AT₁ mRNA expression. This may be due to the duration and dose of Ang II infusion, level of blood pressure, the methodologies used to measure AT₁ mRNA and density, or a combination. It is possible that the direct effects of Ang II on the expression of its receptor may be offset by the ambient blood pressure. It has been shown that infusion of Ang II that did not produce a change in blood pressure did not alter AT₁ mRNA in the aorta and mesenteric arteries. However, pressor infusion of Ang II increased AT₁ mRNA in these vessels. Less is known about the organ-specific regulation of the AT₁ receptor during Ang II infusions that produce hypertension.

Several studies, including our own, have failed to detect changes in AT₁ mRNA levels in the kidney in response to alterations in circulating Ang II levels. This failure to detect changes in the total kidney tissue may reflect opposing changes in glomerular/vascular and tubular AT₁ mRNA, as has been shown in response to low sodium diet and chronic AT₁ receptor blockade in rabbits. However, the present study did not address the regional and segment-specific regulation of intrarenal AT₁ receptor expression during the development of Ang II–induced hypertension.

In response to Ang II, the adrenal gland has been shown to increase Ang II receptors. In the rat adrenal cortex, AT₁A mRNA increased in response to salt depletion, whereas AT₁B mRNA decreased. However, opposite findings have also been reported in the adrenal gland in response to low salt diet. After 2 weeks of Ang II infusion in the rat, Iwai and Inagami found that adrenal AT₁ mRNA levels were elevated but were not altered in the kidney. Our findings are in agreement with most of the studies that reported that the expression of the AT₁ receptor in the adrenal gland is upregulated by its ligand. We found a

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**Figure 5.** Adrenal AT₁A receptor mRNA expression. A, Top, Representative blots showing AT₁A and corresponding GAPDH mRNA levels (lanes 1, 3, and 5, vehicle infused; lanes 2, 4, and 6, Ang II infused). Bottom, Ratio of the densitometric analysis of the autoradiographic signals for AT₁A and GAPDH (n=8) in vehicle-infused (hatched bar) and Ang II–infused (solid bar) rats demonstrated a significant increase in adrenal AT₁A mRNA in hypertensive rats. Bottom, Representative autoradiograph of AT₁A mRNA slot blot hybridized with radiolabeled AT₁A-specific oligonucleotide (lanes 1, 3, and 5, vehicle infused; lanes 2, 4, and 6, Ang II infused). Bottom, Graph plotting the average data for AT₁A mRNA detected by slot blot for vehicle-infused (○; n=8) and Ang II–infused (●; n=6) rats.
modest (~36%) but significant increase in adrenal AT₁ receptor gene expression in Ang II–infused rats (350 ng ⋅ kg⁻¹ ⋅ min⁻¹, 2 weeks) have been reported to be due to increased peripheral metabolism that is independent of elevations in blood pressure.  

In summary, chronic Ang II infusion in the rat that produces a significant elevation in arterial pressure is accompanied by elevations in adrenal AT₁ mRNA levels and the maintenance of kidney and liver AT₁ mRNA and protein levels. The combined effects of elevations in circulating Ang II levels and preservation of the AT₁ receptor in these organs contribute to the hypertensinogenic effects of Ang II.

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