Distribution of angiotensin AT₁ and AT₂ receptor subtypes in the rat kidney

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Miyata, Noriyuki, Frank Park, Xiao Feng Li, and Allen W. Cowley, J r. Distribution of angiotensin AT₁ and AT₂ receptor subtypes in the rat kidney. Am. J. Physiol. 277 (Renal Physiol. 46): F437–F446, 1999.—ANG II contributes importantly to the regulation of renal vascular resistance, glomerular filtration, and tubular epithelial transport, yet there remains a paucity of information regarding the localization of the ANG II type 1 and 2 (AT₁ and AT₂) receptors within the rat kidney particularly within the vasculature. The present study was designed to localize the transcriptional and translational site(s) of AT₁ and AT₂ receptor (AT₁R and AT₂R, respectively) expression within the rat kidney. Using immunohistochemistry, we detected the AT₂R translational sites throughout the kidney, with the strongest labeling found in the vasculature of the renal cortex and the proximal tubules of the outer medulla. The AT₁R protein expression was found throughout the rat kidney, although there was little to no expression found in the glomerulus and medullary thick ascending limbs of Henle (TAL). Gene-specific primers were then designed to distinguish between the receptor subtypes within microdissected renal tubular and vascular segments using RT-PCR. AT₂R, AT₁BR, and AT₂R mRNA were found within the renal vasculature (afferent arterioles, arcuate artery, and outer medullary descending vasa recta). The mRNA for both the AT₁R isoforms was also detected in the glomeruli and the renal tubules (proximal tubules, TAL, and collecting ducts); however, no AT₂R mRNA was detected within the glomerulus and was inconsistently found within the medullary TAL (MTAL). Taken together, these data show that mRNA for the AT₁R subtypes was located in all of the renal tubular and vascular segments. Evidence for AT₂R mRNA was also found in all but two of the vascular and tubular segments, the MTAL, and the glomeruli. These results are consistent with the whole tissue immunohistochemically localized receptors.

TWO TYPES of angiotensin II (ANG II) receptors were pharmacologically described in 1993 and classified as the AT₁ and AT₂ receptors (AT₁R and AT₂R, respectively) (40). More recently, the cDNAs that encode the AT₁ and AT₂ receptors (AT₁AR and AT₁BR, respectively) expression within the rat kidney. Using immunochemical labeling, we detected the AT₁R translational sites throughout the kidney, with the strongest labeling found in the vasculature of the renal cortex and the proximal tubules of the outer medulla. The AT₁R protein expression was found throughout the rat kidney, although there was little to no expression found in the glomerulus and medullary thick ascending limbs of Henle (TAL). Gene-specific primers were then designed to distinguish between the receptor subtypes within microdissected renal tubular and vascular segments using RT-PCR. AT₂R, AT₁BR, and AT₂R mRNA were found within the renal vasculature (afferent arterioles, arcuate artery, and outer medullary descending vasa recta). The mRNA for both the AT₁R isoforms was also detected in the glomeruli and the renal tubules (proximal tubules, TAL, and collecting ducts); however, no AT₂R mRNA was detected within the glomerulus and was inconsistently found within the medullary TAL (MTAL). Taken together, these data show that mRNA for the AT₁R subtypes was located in all of the renal tubular and vascular segments. Evidence for AT₂R mRNA was also found in all but two of the vascular and tubular segments, the MTAL, and the glomeruli. These results are consistent with the whole tissue immunohistochemically localized receptors.

contrast, the coding region of the AT₁R and AT₂R cDNA sequences exhibit only 30% homology (16, 17).

The predominant biological effects of ANG II are believed to be mediated through the stimulation of AT₁R subtypes that mediate vasoconstriction (4), angiotensin (41), and stimulation of sodium and water reabsorption in the proximal tubules (18). To determine the whole tissue distribution of the AT₂R subtypes, several investigators have used RT-PCR (15, 19) and in situ hybridization (22). These studies have demonstrated AT₂R mRNA throughout the kidney with the highest abundance in the outer medulla as determined by in situ hybridization (22) and semiquantitative RT-PCR (19).

At the present time, both the localization and the functional role of each of the isoforms of the AT₁R subtypes within the rat kidney remain unclear. The AT₂R is believed to play the major role in the renal actions of ANG II, but little is known about the intrarenal role of the AT₁BR subtype. Recent studies in the systemic circulation suggest that the AT₁BR may play a role similar to the AT₁A (27), but neither renal vascular nor tubular actions of this receptor isofrom has been explored. Even the localization of the AT₁R isoforms within distinct segments of the renal tubular and vascular system has remained unclear, since it has been difficult to design specific PCR primers for the AT₁R isoforms due to the high degree of homology of the AT₁A and AT₁BR.

A paucity of information also exists about the localization of the AT₂R within the kidney. In situ hybridization studies by Shanmugam et al. (36) initially suggested that the expression of the AT₂R mRNA was developmentally regulated within the rat kidney, since AT₂R mRNA was detectable only in fetal and immature rats. More recently, Ozono et al. (28) reported AT₂R expression in glomeruli and distal tubules of adult rats. In addition, functional studies have provided pharmacological evidence that the AT₂R can influence renal function. Lo et al. (20) observed that the pressure-natriuresis relationship in rats was enhanced by AT₂R inhibition. Other investigators have reported AT₂R involvement in prostaglandin-dependent and -independent vasodilation (13, 38). These studies suggest that AT₂R receptors are present within the adult rat kidney and play a role in influencing renal function.

Because of the limited information regarding ANG II receptor distribution within the rat kidney, the aim of the present study was to design specific PCR primers for each of the ANG II receptor isoforms and localize the site(s) of transcription for the AT₁R (AT₁AR and AT₁BR) and AT₂R by RT-PCR in isolated renal tubules and blood vessels. In addition, the site(s) of translation for
the AT1R and AT2R protein were assessed using immunohistochemical techniques.

**METHODS**

**Experimental Animals**

Male Sprague-Dawley rats weighing 80–120 g (5–6 wk old) from Sasco (Madison, WI), which were given free access to tap water and fed a standard pellet diet (Purina Mills, St. Louis, MO), were used in all studies. All protocols were approved by the Institutional Animal Care Committee, and rats were maintained in the American Association for Accreditation of Laboratory Animal Care approved Animal Resource Center of the Medical College of Wisconsin.

**Protein Isolation and Western Blot Analysis of AT1R and AT2R Antibody**

Rat kidney (n = 3) plasma membrane-enriched fractions were isolated as previously described (30). In brief, kidneys were homogenized, and the homogenate was centrifuged at 3,000 g for 5 min and then at 16,000 g for 20 min. The pellet was resuspended and frozen at 80°C for use in Western blot analysis. A 5-µg (for AT1R) or 25-µg (for AT2R) aliquot was run in parallel with the isolated tubular and microvascular proteins for each blot. The proteins were separated by SDS-PAGE gel and transferred onto a nitrocellulose membrane. Membranes were incubated with the primary antibodies (1:1,000 for the AT1R and 1:2,000 for the AT2R) for 90 min, followed by washes in 5% nonfat milk in TBS-T (30), and then the membranes were washed vigorously. The second antibody was used at a dilution of 1:1,000 and then the blots were stained for horseradish peroxidase activity using enhanced chemiluminescence (Pierce, Rockford, IL) and placed on film.

**Immunohistochemistry for AT1R and AT2R Within the Rat Kidney**

Rat kidneys (n = 6) were fixed in 10% Formalin and subsequently embedded in paraffin. Kidney sections (5 µm) were prepared, and sections were incubated with xylene and hydrated through several washes in ethanol and water to remove the paraffin. Endogenous peroxidase activity was quenched by the addition of 0.3% (vol/vol) hydrogen peroxide in methanol for 10 min. Polyclonal anti-rabbit AT1R (Santa Cruz Biotech, Santa Cruz, CA) or a polyclonal anti-rabbit AT2R antibody [generously provided by Dr. A. S. Greene and described previously by Nora et al. (26)] was used for the immunostaining. The antibodies were used at a dilution of 1:1,000 in 1% BSA in PBS (pH 7.2) and then the kidney sections were incubated for 45 min with the primary antibody at room temperature in a wet chamber. The specificity of the staining was determined by reacting the antibody with a 10-fold (by weight) excess of control antigenic peptide in PBS, which was incubated with the sections for 2 h at room temperature. Secondary antibody (donkey anti-goat) was incubated with the sections for 30 min followed by a complex of streptavidin and biotinylated peroxidase (DAKO, Bucks, UK). The immunoreactivity was detected by the addition of 0.5 mg/ml diaminobenzidine (Sigma, St. Louis, MO) and 0.01% hydrogen peroxide in PBS. Between each step, the sections were washed three times with 200 ml PBS over a period of 15 min. The sections were counterstained with Mayer’s hematoxylin, dehydrated, and mounted.

**Preparation of Glomeruli, Renal Tubules, and Renal Microvessels**

Rats (n = 6) were injected with 5 mg ip furosemide and 30 min later were anesthetized with a mixture of ketamine (50 mg/kg) and acepromazine (5 mg/kg) mixture given intramuscularly. Renal tubules and microvessels were isolated as previously described (30, 42). In brief, the left kidney was perfused with 10 ml dissection solution (DS) prewarmed to 37°C. Following kidney perfusion with DS, 1.0 ml of 2.5% latex-coated blue-dyed microparticles (~1–4 µm in diameter; Polysciences, Warrington, PA) were perfused into the left kidneys. The renal pedicle was ligated, and the kidney was excised and cut into coronal slices (~1–2 mm thick). These tissue slices were placed into DS containing 1 mg/ml collagenase (192 U/mg; Worthington Biochemical, Freehold, NJ), and incubated at 37°C for 35–40 min. For the isolation of inner medullary collecting ducts, the tissue was incubated in collagenase for longer periods (up to 60 min). After collagenase treatment, the tissue slices were rinsed with DS, and placed into a microdissection dish for tubules and microvessel isolation using a Leica M52 stereomicroscope (magnification ×16–100). The following intrarenal blood vessels were dissected from the tissue slices: arcuate artery, afferent arterioles, glomeruli, and outer medullary descending vasa recta (OMDR) from vascular bundles. For the renal tubules, proximal conformed tubules, thick ascending limbs of Henle (TAL), and the collecting ducts (cortex and outer and inner medulla) were isolated as previously described (42). All lengths of the vessels were measured by a calibrated eyepiece micrometer, and for each RT reaction, 40 glomeruli, 40 mm of blood vessels, and 40 mm of renal tubules were isolated. Contaminating debris was rinsed from the dissected vessels, tubules, and glomeruli in a separate wash dish before transfer to an RNase-free ultracentrifuge tube containing 100 µl TRIzol reagent (GIBCO-BRL; Life Technologies, Gaithersburg, MD). The total RNA was extracted and treated with DNase solution as previously described (30). As a negative control for the presence of AT1R and AT2 mRNA, liver mRNA was run in parallel with the isolated tubular and microvascular total RNA.

**Preparation of Oligonucleotide Primers**

All nucleotide primers were purchased from Operon Technologies (Alameda, CA). Oligonucleotide primers were chosen from the published cDNA sequences of angiotensin AT1R (12), AT1B (8), and AT2R (17). The primers for the AT2R spanned both introns found in the genomic DNA sequence, which allowed for differentiation of genomic DNA contamination, but the AT1A and AT1B PCR primers were not intron spanning.

The primer sequence for the angiotensin AT1A corresponded to 5′-CGT CAT CCA TGA CTG TAA AAT TTC-3′ (sense; bp 1097–1120) and 5′-GGG CAT TAC ATT GCC AGT GTG-3′ (antisense; bp 1381–1402). The final PCR product was 306 bp in size.

The primer sequence for the angiotensin AT1B corresponded to 5′-CAT TAT CCG TGA CTG TGA AAT TG-3′ (sense; bp 1369–1391) and 5′-GCT GCT TAG CCC AAA TGG TCC-3′ (antisense; bp 1712–1732). The final PCR product was 344 bp in size.

The primer sequence for the angiotensin AT2R corresponded to 5′-GGG AGC AGC ACA AAG TGG AAA GC-3′ (sense; bp 1586–1608) and 5′-TGC CCA GAG AGG AAG GGT TGC C-3′ (antisense; bp 3332–3353). The final PCR product was 445 bp in size.
Angiotensin II Receptor

Immunohistochemistry of AT1R in the rat kidney. The primary antibody (Fig. 1D) was incubated with the AT1R antibody to demonstrate the specificity of the antibody in the kidney. The antibody was directed against the same receptor sequence used to generate the polyclonal antibody. The specificity of the AT1R antibody was confirmed by comparing the AT1R and AT1R cDNA products generated in the PCR reaction to the corresponding DNA sequences. The optimal PCR primer pairs used in the two experiments were identical (i.e., AT1R and AT1R) and the PCR primers were 100% homologous. The optimal PCR primer pairs used in the two experiments were identical (i.e., AT1R and AT1R) and the PCR primers were 100% homologous.
Microlocalization of AT₁A and AT₁B within the rat kidney. The RT-PCR analysis of the AT₁A and AT₁B mRNA in microdissected tubular and vascular segments is shown in Fig. 3 and summarized in Table 1. The AT₁A and AT₁B mRNA was detected in all of the renal tubular and vascular segments isolated and studied. In summary, both ANG II type 1 receptor isoforms were found in the proximal convoluted tubules, medullary TAL (MTAL), and cortical, outer, and inner medullary collecting ducts. In the renal vasculature, the AT₁A and AT₁B mRNA was observed in the glomeruli, OMDVR, afferent arterioles, and arcuate arteries. As summarized in Table 1, we found positive results (denoted as a “+”) for both AT₁ isoforms in the different segments of the kidney. We performed the RT-PCR assay for the AT₁A and AT₁B in six different segments of the kidney.
rat samples for both the renal vasculature and tubules from the renal medulla to demonstrate consistent localization of the AT1R mRNA; we performed three experiments for the cortical collecting duct and afferent arterioles. It is important to note that the AT1AR and AT1BR mRNA was detected in every rat sample studied. Each experiment was run with tissue controls where we used the adrenal gland and liver (100 ng total RNA) to demonstrate the specificity of the AT1R primers, where the AT1AR was present in both tissues, and only the AT1AR was present in the liver. In addition, contamination was not evident, as demonstrated by the absence of detectable bands in the negative controls: 1) RT-PCR amplification of the DS; and 2) PCR amplification of DNase-treated RNA from the MTAL (MTAL 2 RT) or OMCD (OMCD 2 RT).

Angiotensin AT2 Receptor

Immunohistochemistry of AT2R in rat kidney. The specificity of the AT2R antibody provided by our colleagues Nora et al. was determined in their laboratory by Western blot analysis on membrane-enriched fractions from the kidney (16,000 g pellet), as recently reported (26). Using this AT2R antibody, we found the translational sites of the AT2R to be localized in each of the three regions of the rat kidney, which included the cortex and outer and inner medulla (Fig. 4A). Higher magnification of the renal cortex revealed that the strongest labeling of the AT2R was found within the proximal tubules and distal tubules of the renal cortex, which includes the collecting ducts (Fig. 4B). Little staining was detected in the TAL or in the glomeruli. In the renal medulla, the labeling of the AT2R appeared to be highest in the collecting ducts (Fig. 4C), with very little labeling within the vascular bundles. As a negative control, kidney sections that were incubated with the AT2R antibody preadsorbed with the competing antigenic peptide did not have any labeling, demonstrating the specificity of the binding by the primary antibody (Fig. 4D).

Specificity of the PCR primers for AT2R. The AT2R primers were designed to span both intervening sequences (introns) of the AT2R gene, and the specificity of the AT2R PCR product was verified by PCR sequenc-
Table 1. RT-PCR profile of whole and microdissected renal tissue

<table>
<thead>
<tr>
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<th>AT₁AR</th>
<th>AT₂R</th>
<th>AT₂R</th>
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<tr>
<td>Whole tissue</td>
<td></td>
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<tr>
<td>Adrenal gland</td>
<td>+ (6/6)</td>
<td>+ (6/6)</td>
<td>+ (6/6)</td>
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<tr>
<td>Liver</td>
<td>+ (6/6)</td>
<td>- (0/6)</td>
<td>- (0/6)</td>
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<tr>
<td>Aorta</td>
<td>+ (6/6)</td>
<td>+ (6/6)</td>
<td>+ (6/6)</td>
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<tr>
<td>Microdissected tissue</td>
<td></td>
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<tr>
<td>Proximal tubule</td>
<td>+ (6/6)</td>
<td>+ (6/6)</td>
<td>+ (6/6)</td>
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<tr>
<td>Medullary thick ascending limb</td>
<td>+ (6/6)</td>
<td>+ (6/6)</td>
<td>- (1/6)</td>
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<tr>
<td>Cortical collecting duct</td>
<td>+ (3/3)</td>
<td>+ (3/3)</td>
<td>+ (3/3)</td>
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<tr>
<td>Outer medullary collecting duct</td>
<td>+ (6/6)</td>
<td>+ (6/6)</td>
<td>+ (6/6)</td>
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<tr>
<td>Inner medullary collecting duct</td>
<td>+ (6/6)</td>
<td>+ (6/6)</td>
<td>+ (6/6)</td>
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<tr>
<td>Glomerulus</td>
<td>+ (6/6)</td>
<td>+ (6/6)</td>
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<tr>
<td>Arcuate artery</td>
<td>+ (6/6)</td>
<td>+ (6/6)</td>
<td>+ (6/6)</td>
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<tr>
<td>Afferent arteriole</td>
<td>+ (3/3)</td>
<td>+ (3/3)</td>
<td>+ (3/3)</td>
</tr>
<tr>
<td>Outer medullary descending vasa recta</td>
<td>+ (6/6)</td>
<td>+ (6/6)</td>
<td>+ (6/6)</td>
</tr>
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</table>

Summary of the RT-PCR analyses for the AT₁AR, AT₂R, and AT₂R receptors using extrarenal whole tissue and microdissected renal tubular and vascular segments, showing positive result (+) or negative result (−). Values in parentheses indicate the number of positive results vs. number of experiments performed.

Discussion

The present study was designed to differentiate and localize the angiotensin AT₁R subtypes within the rat kidney. Specific PCR primers were designed to allow for the discrimination of the AT₁AR, AT₂BR, and AT₂R by gel electrophoresis. In addition, specific polyclonal antibodies targeted against the AT₁R and AT₂R were used in immunohistochemistry to demonstrate the translational sites within the kidney.

Localization of the AT₁AR and AT₂BR Within the Rat Kidney

It is well known that ANG II has both vascular and tubular effects within the rat kidney (18, 25, 29, 40). The immunohistochemical results from the present study showed that the AT₁R protein was associated with tubular and vascular structures throughout the kidney, with the strongest labeling being observed in the cortical blood vessels and S3 segment of the proximal tubules in the outer medulla. The results from the present study are consistent with a study by Meister et al. (22) in which the AT₁R mRNA was highly localized to the outer medullary proximal tubules using in situ hybridization. Moreover, binding studies with ¹²⁵I-ANG II in microdissected nephron segments showed binding to be highest in the proximal tubules, with lesser binding in the TAL and collecting ducts (23). Subsequent studies using AT₁R and AT₂R agonist-selective analogs have demonstrated that the majority of the ANG II binding (>80%) within the kidney is to the AT₁R (4, 7, 32, 43). In addition, AT₁R distribution has also been found localized to the interstitial cells of the kidney using ANG II binding techniques (43), but the techniques utilized in this study could not ascertain whether interstitial cells were labeled. In all, neither immunohistochemistry nor ANG II binding studies are able to differentiate between the AT₁R and AT₂R, because of the lack of specific AT₂R subtype analogs and antibodies.

To circumvent this problem, investigators have used RT-PCR to demonstrate the localization of AT₁R subtypes within microdissected renal tubules. Terada et al. (39) observed that the AT₁R mRNA was present throughout the nephron, which included the proximal tubules, TAL, and collecting ducts. However, the PCR primers used in their study were designed within the coding region of the AT₁R where the AT₁AR and AT₂BR have the highest homology (~92% identical) with each other. Subsequent studies by several investigators began to use restriction enzymes following RT-PCR amplification to distinguish the AT₁R isoforms (1). These investigators, however, used this technique only to localize the AT₁R isoforms in different regions of the kidney using whole tissue samples. Recent studies by Bouby et al. (2) demonstrated AT₁AR and AT₂BR mRNA distribution in individual nephron segments, but a number of potential problems could arise from restriction enzyme analysis of PCR products having high homology (greater than 90%), as is the case for the AT₁AR and AT₂R. Specifically, the nucleotide mutations can occur during
PCR product generation so that a particular restriction enzyme site can be added or removed. Additionally, because restriction enzymes recognize unique palindromic sequences, closely homologous mRNAs may not be digested due to the high PCR cycle numbers. This can result in the formation of AT$_{1a}$R:AT$_{1b}$R heteroduplexes, which makes these products unable to be digested. Formation of heteroduplexes is probably the greatest limitation of the restriction enzyme digestion approach (10, 21, 35).

For these reasons, one of the novel contributions of the present study was the development of gene-specific primers that would selectively amplify the AT$_{1}$R subtypes and thus make the restriction enzyme digestion unnecessary to distinguish the AT$_{1a}$R from that of AT$_{1b}$R PCR products. The results of the RT-PCR (as shown in Fig. 2) demonstrated the ability of the present study to design gene-specific primers for highly homologous cDNAs. Further support for the specificity of the PCR primers for selective amplification was obtained.
using different tissue RNAs, which included the adrenal gland, aorta, and liver. It has been previously reported that the adrenal gland has both isoforms of the AT-R (37) as confirmed in our study. The liver, in contrast, yielded only AT1R PCR amplification RNA products, but not AT1BR, which confirms observations by others in rats (1) and mice (3). The results of the present study, therefore, showed that the PCR primers, which were designed for the AT1R and AT1BR, were specific as tested by restriction enzyme digestion and tissue RNA analysis.

The results of our study (summarized in Table 1) demonstrate that all of the tubular segments contain both AT2R and AT1BR receptor isoforms, including the proximal tubules, TAL, and collecting ducts. In the renal vasculature, the AT2R mRNA was also detected in the glomerulus, arcuate artery, afferent arterioles, and OMDVR. Studies by Terada et al. (39) demonstrated AT1R mRNA in the arcuate artery and vascular bundles, but the AT2R PCR primers used in that study could not distinguish between the AT1R subtypes. The physiological role of each of these receptors in each of these distinct segments of renal tubules and vessels remains to be determined.

Localization of AT2R Within Rat Kidney

The functional role of the AT2R has not been fully elucidated within the kidney. It is believed that the AT2R is involved in organogenesis, because of its abundant presence during development (36, 37). Initial studies by Grady et al. (7) found that PD-123319, a specific AT2R antagonist, blocked the binding of [3H]-[Sar1, Ile8]ANG II during embryogenesis and postnatal development of the rat. In situ hybridization studies found that AT2R mRNA was abundantly found in the rat brain, adrenal glands, and lungs throughout development in the rat, but the AT2R mRNA was detected in the rat kidney only for the first 22 days following parturition (36, 37). Studies by others have indicated that less than 20% of the ANG II binding sites in the rat kidney can be attributed to the AT2R (32, 43).

The present study determined that the AT2R mRNA was detected in the adrenal gland and the aorta, but not in the liver. These findings are consistent with previous in situ hybridization and RT-PCR studies (24, 36). In the kidney, we have localized the AT2R mRNA to various tubular and vascular segments from the cortex and medulla, which include the proximal tubules, collecting ducts, arcuate arteries, afferent arterioles, and OMDVR. The presence of AT2R mRNA in afferent arterioles is consistent with the studies of Ruan et al. (32). An absence of AT2R mRNA was observed within the glomerulus and in the MTAL. The absence of glomerular AT2R mRNA in adolescent rats is consistent with previous immunohistochemical (28) and autoradiographic techniques (5) where glomerular AT2R protein was nearly absent in adolescent and adult rats that were fed a normal salt diet. Moreover, the localization of the AT2R mRNA is consistent with the immunohistochemical study performed in the present study, in which we used a specific AT2R antibody. Although the present study demonstrates the presence of the AT2R within the proximal tubules and collecting ducts, further studies need to be performed to address the role(s) of this receptor in the regulation of tubular function.

Functional Roles of the ANG II Receptor Subtypes Within the Renal Vasculature

The role of the ANG II receptor subtypes within the renal vasculature has begun to be elucidated by several investigators. Terada et al. (39) previously demonstrated AT2R mRNA in microdissected arcuate arteries and vasa recta bundles. The transcription of mRNA for both of the AT2R isoforms in the renal vasculature and the supportive immunocytochemical evidence indicates that the vasoconstrictor actions of ANG II on the renal vasculature were related to the stimulation of these receptors. Ikenaga et al. (11), using an isolated split kidney blood perfused juxtedudillary preparation, found that afferent arterioles constricted in the presence of ANG II. The descending vasa recta vessels, which are of particular interest regarding the regulation of medullary blood flow, clearly expressed both isoforms of the AT2R mRNA. The functional significance of these descending vasa recta receptors has been demonstrated by Pallone (29), who has shown that ANG II can cause reductions in luminal diameters of isolated perfused OMDVR through the stimulation of an AT2R. The ability of ANG II to constrict these descending vasa recta could be blocked by the addition...
of saralasin, an AT₁R antagonist. Although isoform-specific AT₁R antagonists do not currently exist to determine the functional roles of these receptors, it is evident from the present study that both of these isoforms are widely expressed throughout the renal vasculature and tubules, which may be of functional or pharmacological significance.

With regard to the AT₂R, the present study has demonstrated that the AT₂R mRNA and protein are present within different segments of the cortical and medullary vasculature, including the arcuate artery, afferent arterioles, and OMDVR. The finding of the AT₂R within renal vessels is consistent with the previous observation by Ruan et al. (32). The role of the AT₂R in the renal vasculature remains to be determined but on a whole tissue basis may have a role in the stimulation of nitric oxide (38). Future studies will need to be performed to address the role of the AT₂R within the kidney, particularly within the renal vasculature.

In summary, the present study has demonstrated that AT₁R mRNA and protein is found throughout the kidney, particularly within the renal vasculature. AT₂R mRNA and protein was also found widely distributed in the various tubular and vascular segments of the renal cortex and medulla. AT₂R mRNA was localized to the proximal tubules, collecting ducts, arcuate arteries, afferent arterioles, and OMDVR. The results indicated that the AT₂R mRNA was not readily detectable in the glomerulus or the TAL. It is important to note that in the present study we used techniques which determined the qualitative distribution of the AT₁R and AT₂R but that future studies need to be performed to quantitate and demonstrate their functional roles within distinct segments of the renal vasculature and tubules.

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