Roles of AT₁ and AT₂ Receptors in the Hypertensive Ren-2 Gene Transgenic Rat Kidney

Jialong Zhuo, Mitsuru Ohishi, Frederick A.O. Mendelsohn

Abstract—Adult Ren-2 gene transgenic rats, TGR(mRen-2)27, exhibit elevated circulating and kidney angiotensin II (Ang II) levels in the presence of severe hypertension. The aim of this study was to examine whether AT₁ and AT₂ receptors in the kidney and renal hemodynamic and tubular responses to blockade of these receptors were altered in the Ren-2 gene transgenic rats during the maintenance phase of hypertension. Renal AT₁ and AT₂ receptors were mapped by in vitro autoradiography (n=8), and the effects of blockade of these receptors on mean arterial pressure (MAP), heart rate (HR), and renal cortical (CBF) and medullary blood flows (MBF) were studied in anaesthetized, adult age-matched male homozygous TGR rats (n=12) and Sprague-Dawley (SD) rats (n=7). TGR rats showed higher basal MAP (P<0.001), heart and kidney weight (P<0.001), plasma renin activity (P<0.05) and plasma Ang II level (P<0.05), and CBF (P<0.05) and MBF (P<0.05) than SD rats. AT₁ receptor binding was significantly increased in the glomeruli, proximal tubules, and the inner stripe of the outer medulla of TGR rats (P<0.01), while the AT₂ receptor binding was low at all renal sites of TGR and SD rats. Immunohistochemistry revealed that this increased AT₁ receptor labeling occurred mainly in vascular smooth muscle layer of intrarenal blood vessels including afferent and efferent arterioles, juxtaglomerular apparatus, glomerular mesangial cells, proximal tubular cells, and renomedullary interstitial cells (RMICs) in the transgenic rats. Blockade of AT₁ receptors with losartan in TGR rats markedly reduced MAP to the normotensive level (P<0.001) without altering HR. Both CBF (P<0.005) and MBF (P<0.05) were significantly increased by losartan in the transgenic rats. By contrast, losartan only caused a smaller decrease in MAP and an increase in renal CBF in SD rats (P<0.05), PD 123319 was without any renal effect in both SD and TGR rats. These findings suggest that markedly increased AT₁ receptors in renal vasculature, glomerular mesangial cells, and RMICs in the presence of fulminant hypertension and elevated circulating and tissue Ang II levels may play an important role in the maintenance of hypertension in the Ren-2 gene transgenic rats. (Hypertension. 1999;33[part II]:347-353.)

Key Words: angiotensin II receptor, angiotensin II Ren-2 gene renal cortical and medullary blood flows

The transgenic TGR(mRen-2)27 (TGR) rat harboring the murine Ren-2 gene is characterized by development of fulminant hypertension with extensive tissue injury and organ dysfunction in the brain, heart and blood vessels, and kidney.¹⁻³ The development and maintenance of severe hypertension in TGR rats appear to be angiotensin II (Ang II)–dependent, as both angiotensin-converting enzyme inhibitors and the angiotensin type 1 (AT₁) receptor antagonists reduce blood pressure to levels seen in their normotensive counterparts, Sprague-Dawley (SD) rats.⁴⁻⁶ Because renin and Ang II are suppressed in plasma and the kidney, it has been suggested that overexpression of the Ren-2 gene in extrarenal tissues, including adrenal glands, the heart and blood vessels, and the brain, largely contributes to the development and maintenance of hypertension in the Ren-2 gene transgenic rats.¹⁻³⁻⁷

However, recent evidence suggests that the abnormal regulation of local angiotensin formation in the kidney also plays an important role in the pathogenesis of severe hypertension in TGR rats.⁸⁻¹⁰ Renal Ang II appears to mediate the marked right shift of pressure natriuresis response curve to a higher arterial pressure⁹ and to the enhanced tubuloglomerular feedback responsiveness in TGR rats.¹¹ Moreover, despite severe hypertension and suppressed renal renin expression, the levels of plasma and kidney Ang II have been reported to be normal¹⁰ or even markedly elevated⁶ in TGR rats. These observations indicate that intrarenal Ang II formation and its receptors in these transgenic rats are not under an appropriate negative feedback regulation.

The primary aim of this study therefore was to examine whether there was an abnormal change of AT₁ and AT₂ receptors or altered renal hemodynamic and excretory responses to blockade of these receptors in anesthetized TGR(mRen-2)27 hypertensive rats compared with their normotensive counterparts, transgene-negative SD rats.

Methods

Animals
Twenty male adult (~12 weeks old), age-matched homozygous transgenic Ren-2 hypertensive rats and 15 normotensive SD rats
were used in the present study. TGR rats, which were initially obtained from Dr D. Ganten of the Max-Delbruch Center for Molecular Medicine, Berlin-Buch, Germany, are now maintained in the Department of Physiology, The University of Melbourne. Homozygous TGR rats are routinely treated with lisinopril to control hypertension, but in this study to exclude the influences of ACE inhibition on circulating and tissue renin-angiotensin systems (RAS) as well as on renal function, lisinopril was withdrawn 3 weeks before experiments were performed. Both TGR and SD rats were maintained on a normal rat diet and allowed free access to tap water. This study was approved by the Animal Experimental Ethics Committee of the Howard Florey Institute of Experimental Physiology and Medicine.

Measurement of Plasma Renin Activity, Ang I, and Ang II

Plasma levels of the components of the circulating RAS were measured by radioimmunoassay as described.12 Briefly, after decapitation, 2 mL of trunk blood samples were collected from TGR (n=8) and SD (n=8) rats into chilled tubes containing heparin for assays of plasma renin activity (PRA) or into tubes containing a BAL/EDTA mixture for radioimmunoassays of plasma Ang I and Ang II.12 Blood samples were then centrifuged at 3000 g for 15 minutes at 4°C, and the plasma was collected and stored at −80°C for later radioimmunoassays.12

Measurement of AT1 and AT2 Receptors by Quantitative In Vitro Autoradiography

Measurement and characterization of renal AT1 and AT2 receptors were performed using in vitro autoradiography and the radioligand [125I]-[Sar1,Ile8]Ang II (Peninsula Laboratories), as described previously.16 Frozen kidney sections (20 μm thick) of TGR and SD rats were first preincubated in 10 mmol/L sodium phosphate buffer, pH 7.4, for 15 minutes to remove endogenous Ang II, which binds to its receptors. The sections were then incubated for 1 hour in the same fresh buffer containing ~100 pmol/L [125I]-[Sar1,Ile8]Ang II at 22°C. In all cases, both radioligand and agonist or antagonists were added into the buffer simultaneously. Nonspecific binding was determined in parallel incubations containing an excess concentration (1 μmol/L) of unlabeled Ang II (Hypertensin, Ciba Pharmaceuticals). AT1 receptors were determined in the presence of 10 μmol/L of the AT2 receptor antagonist PD 123319, while AT2 receptors were measured in the presence of 10 μmol/L of the AT1 receptor antagonist losartan. Binding specificities of AT1 and AT2 receptors were examined in duplicate binding competition experiments, in which various concentrations of unlabeled Ang II, losartan, and PD 123319 were added into the incubation buffer.13 After incubation and subsequent washes, the sections were loaded into x-ray cassettes together with a set of [125I]-radioactivity standards and exposed to x-ray films (Agfa-Gaevert) for 7 days. The films were then developed and autoradiographs analyzed by computerized densitometry (MCID, Imaging Research Inc) as described.13

Cellular Localization of AT1 Receptor Labeling by Immunohistochemistry

To provide cellular localization of AT1 receptors in TGR and SD rat kidneys, immunohistochemistry was performed using a polyclonal antibody generated against the amino acid sequence 15-24, Ac-CQDDCPKAGRHC-NH2, of the human AT1 receptor as described previously.14 Serial frozen sections, 8 μm thick, from SD and TGR rats were incubated overnight with the primary antibody at 4°C for positive controls, and nonimmune rabbit serum was used on adjacent sections as negative controls. The labeled streptavidin-biotin complex system with 3-amino-9-ethylcarboxazole development was used as described.15 All sections were stained with hematoxylin and eosin before histological examination.

Effects of AT1 and AT2 Receptor Blockade on Renal Cortical and Medullary Perfusion

Because receptor mapping experiments revealed significant increases in AT1 receptors in the cortex and the inner stripe of the outer medulla of TGR rats compared with normotensive SD rats, renal cortical and medullary hemodynamic and tubular secretory responses to the AT1 receptor antagonist losartan (a gift of DuPont Merck Pharmaceuticals Co) and the AT2 receptor antagonist PD 123319 (a gift of Parke-Davis) were compared in separate groups of anesthetized TGR and SD rats (n=7) using a laser-Doppler flowmeter as described.16 All rats were anesthetized with pentobarbitone (Nembutal, 100 μg/kg IP) and prepared for a standard renal clearance experiment.17 The right jugular vein was cannulated for infusions of saline, clearance markers, and drugs, while the right carotid artery was cannulated for blood sampling and for monitoring blood pressure. The left kidney was exposed through a left flank incision and placed in a micropuncture cup. Laser-Doppler probes were inserted into the cortex (~2 mm deep) and into the inner stripe of the outer medulla (~5 mm deep), respectively, for monitoring renal cortical and medullary blood flows using a laser-Doppler flowmeter (LoLAB laser Doppler, Moor Instruments Ltd). Arterial blood pressure, heart rate, and renal cortical and medullary blood flows were continuously recorded using an 8-channel PowerLab data acquisition system interfaced with a Microsoft Work Station 4.0 (AD Instruments Ltd). The hemodynamic variables were analyzed using the Chart for Windows Data Analysis System.

On completion of surgical procedures, animals were infused intravenously with saline at 30 μL/min containing 1% para-aminomphenic acid (PAH, Sigma) and 8% inulin for estimation of whole-kidney renal plasma flow (RPF) and glomerular filtration rate (GFR), respectively, as described.17 The rats were allowed to stabilize for at least 60 minutes before they were subjected to the following protocol: ~30 minutes recordings under basal conditions, followed by two further 30-minute periods after administration of the AT1 receptor antagonist PD 123319 (5 mg/kg bolus followed by constant infusion of 50 μg/kg per minute IV); the AT1 receptor antagonist losartan was then added to the infusion (5 mg/kg bolus followed by constant infusion of 50 μg/kg per minute IV). The chosen doses of PD 123319 and losartan have been shown to completely abolish AT1 and AT2 receptor binding in the rat kidneys and adrenal glands18 or to inhibit Ang II–induced pressor and renal hemodynamic responses after intravenous administration.17 An additional group of TGR rats (n=5) were infused with saline and clearance markers only throughout the experiment for time control. Arterial pressure, heart rate, and renal cortical and medullary blood flows were continuously recorded throughout the experiment. Arterial blood samples and urine samples were collected through the carotid arterial catheter and a bladder catheter, respectively.

Data Analysis and Statistics

Data are presented as the mean±SEM. Urine volume was determined gravimetrically, and concentrations of sodium and potassium in plasma and urine were measured by flame photometry (model IL943, Instrumentation Laboratories). Whole-kidney RPF was estimated by PAH clearance, while GFR was measured by inulin clearance as described.17 The differences between experimental periods within the group were compared using 1-way ANOVA with repeated comparisons (Tukey test). The differences between TGR rats and their time controls, and differences between TGR rats and SD rats on the same corresponding parameters, or experimental periods, were analyzed using Student’s unpaired t test. P<0.05 was considered statistically significant.

Results

Basal Cardiovascular Variables and Circulating RAS Activity in TGR and SD Rats

Homozygous TGR rats showed markedly elevated mean arterial blood pressure (SD, 121±7 mm Hg; TGR, 187±7 mm Hg; P<0.001), while there was no difference in heart rate between the 2 strains. Although body weight was similar, TGR rats had significant increases in heart weight (SD, 1.16±0.04 g; TGR, 1.61±0.05 g; P<0.001), and kidney
weights (SD, 1.12±0.04 g; TGR, 1.62±0.02 g; P<0.001). Plasma renin activity (SD, 3.2±0.3 ng Ang I/mL/h; TGR, 7.9±0.8 ng Ang I/mL/h; P<0.01) and plasma Ang I (SD, 0.5±ng/mL; TGR, 2.8±0.4 ng/mL; P<0.01) and Ang II (SD, 15±3.6 pg/mL; TGR, 263±35 pg/mL; P<0.01) levels were significantly higher in TGR rats than in SD rats.

**Figure 1.** In vitro autoradiographic and immunohistochemical localization of AT₁ receptors in SD (A) and TGR(mRen-2)27 (B) rat kidneys. C, AT₁ receptor protein labeling in a TGR rat kidney. AA indicates afferent arteriole; C, cortex; EA, efferent arteriole; G, glomerulus; IM, inner medulla; IS, inner stripe of the outer medulla; JGA, juxtaglomerular apparatus; M, mesangial cell; and P, proximal tubule. C, Original magnification X300.

**Figure 2.** Comparison of quantitative autoradiographic data on AT₁ receptor binding in the glomerulus (GLO), proximal tubule (PCT), and inner stripe of the outer medulla (ISOM) in SD rats and TGR(mRen-2)27 rats. *P<0.05, **P<0.01; ***P<0.001, compared with SD rats.

**AT₁ and AT₂ Receptor Binding in the Kidneys of TGR and SD Rats**

As expected, Ang II receptors in the kidneys of both TGR rats and SD rats were predominantly of the AT₁ subtype, while AT₂ receptor binding was very low to undetectable in both strains. The distribution and quantification of AT₁ receptors in the kidneys of both SD rats and TGR rats are shown in Figures 1 and 2, respectively. Despite severe hypertension and increased plasma Ang II concentration in TGR rats, AT₁ receptor binding at all sites in the kidney, including glomeruli, proximal tubules, and the inner stripe of the outer medulla, was significantly higher in TGR rats compared with that in SD rats (Figures 1B and 2). In TGR rats, immunohistochemistry shows strong AT₁ receptor labeling on vascular smooth muscle cells of large and small intrarenal blood vessels including afferent and efferent arterioles and juxtaglomerular apparatus, and moderate labeling in glomerular mesangial cells adjacent to the vascular pole and in proximal tubular epithelia (Figure 1C). A similar pattern of AT₁ receptor distribution also occurred in the SD rat kidney, but it was not possible to quantify the differences in AT₁ receptor protein by immunohistochemistry.

Binding competition experiments in vitro revealed higher inhibitory potencies of the AT₁ receptor antagonist losartan to displace 125I-[Sar⁸,Ile⁸]Ang II binding in the glomeruli and the inner stripe of the outer medulla of TGR rats than those of SD rats (P<0.05). 125I-[Sar⁸,Ile⁸]Ang II binding curves in the presence of losartan were about one log-order more to the left in TGR rats than in SD rats (not shown). However, the AT₂ receptor antagonist PD 123319 was without any effect on 125I-[Sar⁸,Ile⁸]Ang II binding at concentrations as high as 10μmol/L.
Cardiovascular Responses to Blockade of AT_2 Receptors

In SD rats, blockade of the AT_2 receptors with PD 123319 had no effect on MAP, whereas the same dose of losartan markedly decreased MAP by \( \approx 17 \) mm Hg (\( P<0.05 \); Figure 3). In TGR rats, PD 123319 caused a slight but not significant fall in MAP (PD, 170±10 mm Hg; NS), whereas losartan markedly decreased MAP by >55 mm Hg to a level similar to basal MAP observed in SD rats (128±6 mm Hg; \( P<0.001 \)). Heart rate was not altered by PD 123319 or losartan in either SD rats or TGR rats. There were no significant changes in MAP and heart rate in TGR time controls.

Cortical and Medullary Hemodynamic and Tubular Responses to Blockade of AT_1 and AT_2 Receptors

Basal renal functional parameters in TGR and SD rats are shown in the Table. Compared with SD rats, TGR rats had significantly higher basal urine flow rate, urinary sodium excretion, and GFR and RPF (Table). TGR rats also showed higher renal cortical (CBF) and inner stripe of outer medullary blood flows (MBF) than SD rats as measured by laser-Doppler flowmetry (Table). Blockade of the AT_2 receptor with PD 123319 did not significantly alter either cortical or medullary perfusion in either strain (Figure 3). By contrast, AT_1 receptor blockade with losartan caused significant increases in CBF by 20% and 30% in SD and TGR rats, respectively, whereas MBF was increased only in TGR rats (Figure 3). Interestingly, losartan increased whole-kidney GFR and RPF and enhanced urinary water and sodium excretion in SD rats, but it significantly reduced GFR and urinary sodium and potassium excretion without altering RPF and urine flow rate in TGR rats (Table). In TGR time controls, all renal hemodynamic and tubular functional parameters remained unaltered throughout the experiment.

Discussion

Most previous studies in TGR(mRen-2)27 rats have focused on the roles of extrarenal tissue RAS in the development and maintenance of hypertension in this experimental model of genetic hypertension.\(^1,2\) Although suppression of plasma and kidney renin was initially reported in the Ren-2 gene transgenic rats,\(^1,2\) others found that the levels of plasma and kidney Ang II were not as low as initially indicated, or were even markedly elevated, at least in homozygous TGR rats.\(^8,10,20\) High concentrations of plasma Ang II have been shown in trunk blood samples of decapitated TGR rats\(^20\) and in anesthetized TGR rats surgically prepared for renal micropuncture experiments.\(^10\) Recently, Campbell et al\(^8\) systematically compared the circulating and tissue levels of major components of the RAS including kidney, adrenal, heart, blood vessels, and brain in TGR and SD rats. Plasma levels of active renin and Ang II in that study were 4-fold higher in TGR rats compared with normotensive SD rats, and kidney Ang II level was also increased by 2- to 4-fold in TGR rats.\(^8\) Although other studies reported normal Ang II levels in whole-kidney tissue or in proximal tubular fluid of TGR rats compared with transgene-negative SD rats, the levels of intrarenal Ang II were considered inappropriately high in the presence of hypertension and suppressed kidney renin.\(^10,20\) In agreement with the findings of these studies, we found that in trunk blood samples collected after decapitation, plasma renin activity was 2.5-fold higher, and plasma levels of Ang I and Ang II were 5- and 17-fold higher in TGR rats than in SD rats.

Figure 3. MAP and renal cortical and medullary blood flow responses to blockade of the AT_2 receptor with PD 123319 and the AT_1 receptor with losartan in anesthetized SD rats and TGR(mRen-2)27 rats. *\( P<0.05 \); **\( P<0.01 \), compared with control period within the group; #\( P<0.05 \), ##\( P<0.001 \), compared with corresponding periods in SD rats.
respectively. While it is likely that prorenin activation during collection of trunk blood samples might partly contribute to higher levels of circulating Ang II in our TGR rats, Campbell and coworkers, who used the same TGR rats maintained in the same laboratory, found that this in vitro activation of prorenin contributed little to several-fold increases in plasma renin and angiotensin peptides in their TGR rats. Other factors such as the severity of hypertension might have contributed to these different findings. Nevertheless, because samples were collected and assayed in a similar way for TGR rats and SD rats, our findings, as well as those of others, suggest that increased circulating and renal angiotensins in the presence of persistent, severe hypertension may indeed play an important role in the pathogenesis of hypertension in the Ren-2 gene transgenic rats.

One of the interesting findings in the present study is that despite fulminant hypertension accompanied by increased plasma levels of renin and Ang II, AT₁ receptor binding was significantly increased in the kidneys of TGR rats compared with those of SD rats as measured by quantitative in vitro autoradiography (Figures 1 and 2). Increased AT₁ receptors occurred at all anatomic sites, namely the glomeruli, the interglomerular region corresponding to proximal tubules, and the inner stripe of the outer medulla. Further immunohistochemistry revealed strong AT₁ receptor labeling in vascular smooth muscle cells of large and small intrarenal blood vessels including afferent and efferent arterioles, juxtapglomerular apparatus, glomerular mesangial cells particularly close to the vascular pole of the glomerulus, and in proximal tubules (Figure 2). However, we found no significant AT₂ receptor binding at any of the renal sites in either strain, consistent with our previous findings in normal rats. This upregulation of AT₁ receptors in the glomeruli and the inner stripe of the outer medulla also occurs in the kidney of spontaneously hypertensive rats (J.Z., unpublished observations, 1998) but has not been reported in the kidney of TGR rats previously. In contrast, aortic and cardiac AT₁ receptor mRNA expression has been reported to be downregulated in TGR rats.

The increase in AT₁ receptors in the TGR rat kidney in the present study was unexpected and intriguing given the fact that these rats exhibited higher circulating and intrarenal Ang II levels. It is well documented that AT₁ receptors in blood vessels and the kidney are under negative feedback regulation by Ang II: high circulating and renal Ang II levels downregulate, whereas low concentrations of plasma and renal Ang II upregulate AT₁ receptors in vascular smooth muscle cells, glomerular mesangial cells, and renomedullary interstitial cells (RMICs) of the kidney. By contrast, AT₁ receptor mRNA expression appears to be upregulated by high levels of Ang II in isolated or cultured proximal tubular cells. The negative feedback–regulating mechanism of AT₁ receptors in vascular smooth muscle cells, mesangial cells, and RMICs may be important in maintaining normal blood pressure and body salt and fluid homeostasis. Our results indicate that TGR rats are unable to respond effectively to increased circulating and tissue Ang II levels to reduce AT₁ receptors and/or AT₁ receptor response to Ang II in the presence of severe hypertension. Such an abnormal regulation of kidney AT₁ receptors may therefore contribute at least partly to the maintenance of fulminant hypertension in TGR rats.

In support of the in vitro findings, renal AT₁ receptors in the TGR rats responded markedly to the AT₁ receptor
suggest that AT2 receptors exert a weak depressor role in TGR rats after PD 123319 and hypertonic saline infusion either TGR or SD rats, MAP was significantly increased in hypertensive Ren2 transgenic rats and their normotensive counterparts. In our study, AT2 receptors exerted no significant influences in MAP and induced renal cortical vasodilatation, the extents of these effects were much smaller in normotensive SD rats (Figure 3). Although laser-Doppler flowmeters are widely used to monitor local tissue perfusion in the kidney, some caution should be taken to interpret the hemodynamic data as measured by this technique because many factors other than red blood cell velocity also influence the absolute voltage signals. However, basal RPF, expressed as gram of kidney weight per minute, was also higher in TGR rats than in SD rats in the present study. Furthermore, we found that changes in renal cortical and medullary perfusion after losartan infusion, expressed as percentage of increase from baseline, were also proportional to those of the absolute voltage signals in the present study. Thus, our data, obtained under similar experimental conditions and protocols, are consistent with the proposition that basal renal cortical and medullary perfusions were higher and their subsequent responses to AT1 receptor blockade were increased in the Ren-2 gene transgenic rats. Interestingly, unlike SD rats in which losartan increased whole-kidney GFR and RPF and induced diuresis and natriuresis,17 blockade of the AT1 receptors with losartan significantly reduced GFR and urinary sodium and potassium excretion in TGR rats without altering RPF and urine flow rate (Table). These observations are in good agreement with previous studies in the contralateral kidney of 2-kidney, 1 clip Goldblatt hypertensive rats26 and in the kidney of TGR rats, in which antidiuresis and antinatriuresis occurred, or GFR fell, after captopril or losartan treatment.4,27 Because GFR and sodium and water excretion fell in parallel with a marked fall in MAP, these effects were most likely pressure-dependent in TGR rats.5,9 Similarly, the observed higher basal renal hemodynamic and excretory function in TGR rats than in SD rats (Table) was also probably the result of severe hypertension and increased renal perfusion pressure.

The present study was also designed to evaluate whether AT1 receptors play functional roles in the regulation of renal hemodynamics and urinary water and electrolyte excretion in TGR hypertensive rats and their normotensive counterparts. In our study, AT1 receptors exerted no significant influences in MAP and renal hemodynamic and tubular excretory responses in either TGR or SD rats (Figure 3). These findings were consistent with receptor binding data which showed a very low level of renal AT2 receptors in these animals. Recently, Nishioka et al19 reported that although blockade of AT2 receptors with PD 123319 did not alter basal MAP in either TGR or SD rats, MAP was significantly increased in TGR rats after PD 123319 and hypertonic saline infusion were administered simultaneously. These findings might suggest that AT2 receptors exert a weak depressor role in TGR rats.19 However, we did not observe a rise in MAP after PD 123319 infusion in either SD rats or TGR rats. The reasons underlying these different conclusions are not known, but the differences in phenotypes of the transgenic rats and in experimental protocol used might account for the variance. Basal MAP was much lower in TGR rats of that study (<120 mm Hg)19 than in those of the present study (~190 mm Hg). Other factors (such as whether conscious19 or anesthetized rats were used and whether the rats were infused with isotonic or hypertonic saline19) might have also contributed to the differences in blood pressure response to PD 123319. However, our receptor mapping studies detected no significant levels of AT1 receptors in either TGR or SD rat kidney, nor did we observe an inhibitory effect of PD 123319 on displacing [125I]-[Sar1,Ile8]Ang II binding even at concentrations as high as 10 μmol/L, in competition binding experiments. Therefore, our findings suggest that AT2 receptor probably plays only a minor, if any, physiological role in the regulation of renal hemodynamics and tubular excretory function, although AT2 receptors appear to mediate nitric oxide production in the kidney of conscious SD rats.28

In summary, the present study demonstrates that AT1 receptors were significantly increased in intrarenal blood vessels, glomerular mesangial cells, juxtaglomerular apparatus, proximal tubules, and renomedullary interstitial cells of the Ren-2 gene transgenic rat kidney. Moreover, in vivo blockade of the AT1 receptor with losartan produced a more pronounced antihypertensive effect and increases in renal cortical and medullary perfusion in these transgenic animals. Although the role of overexpression of the Ren-2 gene in extrasrenal tissues in the development and maintenance of hypertension in this model is well recognized, our results suggest that increased AT1 receptors in multiple renal cells in the presence of fulminant hypertension and activated circulating and tissue RAS may play an important role in the pathogenesis of hypertension in these rats. Because the present study was performed only in adult transgenic rats (>10 weeks old) with established phase of hypertension, increased AT1 receptors in the Ren-2 gene transgenic rat kidney therefore may occur only during the maintenance phase of hypertension. Whether increased AT1 receptors also occur in young Ren-2 gene transgenic rat kidney (<5 weeks old) during the development phase of hypertension remains to be further studied.

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