Oxidative stress and glomerular filtration barrier injury: role of the renin-angiotensin system in the Ren2 transgenic rat

Adam T. Whaley-Connell, Nazif A. Chowdhury, Melvin R. Hayden, Craig S. Stump, Javad Habibi, Charles E. Wiedmeyer, Patricia E. Gallagher, E. Ann Tallant, Shawna A. Cooper, C. Daniel Link, Carlos Ferrario, and James R. Sowers. Oxidative stress and glomerular filtration barrier injury: role of the renin-angiotensin system in the Ren2 transgenic rat. Am J Physiol Renal Physiol 291: F1308–F1314, 2006. First published June 20, 2006; doi:10.1152/ajprenal.00167.2006.—TG(mRen2)27 (Ren2) transgenic rats overexpress the mouse renin gene, manifest hypertension, and exhibit increased tissue ANG II levels and oxidative stress. Evidence indicates that elevated tissue ANG II contributes to oxidative stress, increases in glomerular macromolecular permeability, and consequent albuminuria. Furthermore, angiotensin type 1 receptor (AT1R) blocker reduces albuminuria and slow progression of renal disease. However, it is not known whether improvements in glomerular filtration barrier integrity and albuminuria during treatment are related to reductions in oxidative stress and/or kidney renin-angiotensin system (RAS) activity. To investigate the renal protective effects of AT1R blockade, we treated young (6–7 wk old) male Ren2 rats with valsartan (Ren2-V; 30 mg/kg) for 3 wk and measured urine albumin, kidney malondialdehyde (MDA), RAS component mRNAs, and NADPH oxidase subunits (gp91phox and Rac1) compared with age-matched untreated Ren2 and Sprague-Dawley (S-D) rats. Basement membrane thickness, slit pore diameter and number, and foot process base width were measured by transmission electron microscopy (TEM). Results indicate that AT1R blockade lowered systolic blood pressure (30%), albuminuria (91%), and kidney MDA (80%) in Ren2-V compared with untreated Ren2 rats. Increased slit pore number and diameter and reductions in basement membrane thickness and podocyte foot process base width were strongly associated with albuminuria and significantly improved following AT1R blockade. AT1R blockade was also associated with increases in angiotensin-converting enzyme-2 and nephrilysin expression, demonstrating a beneficial shift in balance of renal RAS. Thus reductions in blood pressure, albuminuria, and tissue oxidative stress with AT1R blockade were associated with improved indexes of glomerular filtration barrier integrity and renal RAS in Ren2 rats.

TG(mRen2)27 rat; malondialdehyde; NADPH oxidase; angiotensin receptor blocker

ANGIOTENSIN II (ANG II) activation of the angiotensin type 1 receptor (AT1R) leads to deleterious effects such as oxidative stress, inflammation, and endothelial dysfunction, manifesting as hypertension (HTN), albuminuria, and progressive renal dysfunction, that may ultimately lead to chronic kidney disease (CKD) (6, 12, 13, 15, 27, 28, 33). Treatment with blockers of the renin-angiotensin system (RAS) decreases progression of albuminuria and renal dysfunction, especially in diabetic nephropathy (31). A rodent model to study increased RAS activity and elevated tissue levels of ANG II is the hypertensive, insulin-resistant TG(mRen2)27 (Ren2) transgenic rat (3–5, 14, 23, 34). Research conducted in this and other proteinuric models has shown that ANG II-induced oxidative stress contributes to abnormalities of glucose metabolism, HTN, and albuminuria associated with glomerulosclerosis (3–5, 14, 20, 23, 34) and that these effects can be abrogated by AT1R blockade (3, 24, 34).

Evidence for a local RAS in the glomerulus raises the prospect of ANG II-induced podocyte and filtration barrier injury (1, 11). Furthermore, in vitro protein exposure, mechanical stretch, and glomerular HTN have also been shown to enhance tissue ANG II production, which may potentiate the impact of elevated blood pressure on glomerular injury (1, 12, 16, 32). Previous work related to the pathogenesis of albuminuria has delineated abnormalities such as basement membrane thickening, loss of the slit pore diaphragm integrity, and widening of the podocyte foot process base width (16, 32). Characteristic changes of the filtration barrier and podocytes are best evaluated at the ultrastructural level, using transmission electron microscopy (TEM), but there has been little research addressing the impact of AT1R blockers on ANG II- and HTN-mediated glomerular injury at the TEM level (21). AT1R blockers (ARBs) may also improve renal function through effects on angiotensin-converting enzyme-2 (ACE2), a carboxypeptidase similar in size and sequence to ACE, since a previous study showed upregulation of renal cortex ACE2 following blockade of the systemic RAS (9). Studies have confirmed the role of ACE2 in hydrolyzing ANG II to ANG-(1–7), a peptide that generally counterbalances the effects of ANG II (29, 30). Moreover, recent evidence indicates that AT1R blockade augments ACE2 activity (8, 10). Because the interplay between ACE2 and the RAS may be critical in the regulation of renal function, the current study investigated the impact of AT1R blockade on podocyte and filtration barrier changes in a hypertensive rodent model displaying albuminuria and increased tissue ANG II levels (3–5, 14, 23, 34). Indexes

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of oxidative stress (malondialdehyde, NADPH oxidase subunits) and RAS activity (ACE2, ACE, and nephrilysin mRNAs) in kidney tissue were also measured in the presence or absence of AT1R blockade.

**METHODS**

### Animals and Treatments

Male transgenic Ren2 rats and male Sprague-Dawley (S-D) controls were received at 4–5 wk of age from Wake Forest University School of Medicine. All animal procedures were approved by the University of Missouri and Harry S. Truman Veterans Affairs Medical Center Institutional Animal Care and Use Committees and housed in accordance with National Institutes of Health (NIH) guidelines. Ren2 rats (6–7 wk of age) were randomly assigned to untreated or valsartan-treated (Ren2-V) groups. Age-matched S-D rats were studied in parallel. Ren2-V animals received valsartan (30 mg·kg⁻¹·day⁻¹) in their drinking water for 21 days. Ren2 and S-D animals received drinking water that was of equal pH as that of the Ren2-V rats.

### Blood Pressure

Systolic blood pressure (SBP) was measured in triplicate using the tail-cuff method (Harvard Systems, Student Oscillometric Recorder) before treatment and after 3 wk of treatment with valsartan or under control conditions.

### Urine Albumin

Urine albumin was determined using a commercially available kit specific for rat urine albumin (Nephrat; Exocell, Philadelphia, PA). Urine was collected over a 16-h period at the end of treatment and normalized to creatinine, which was measured by Jaffé reaction on an automated chemistry analyzer (Olympus AU400; Olympus America, Dallas, TX).

### Tissue Malondialdehyde

Butylated hydroxytoluene (BHT; 5 mM) was added to kidney tissue (100 mg) to prevent new lipid peroxidation. Samples were then homogenized in a buffer solution (0.25 M sucrose, 0.5 mM EDTA, 50 mM HEPES, protease inhibitors (Complete, Roche Diagnostics), phosphate inhibitors (Cocktail-1 and -2, Sigma Chemical)) and centrifuged at 1,000 g for 5 min. The supernatant was collected for malondialdehyde (MDA) and O-phthalaldehyde (OPA) spectrophotometric assay kit (OxisResearch Biotech, Portland, OR). MDA concentrations were measured using an MDA-586 fluorometric assay and FLX-800 fluorometer (BioTek, Winooski, VT).

### Immunofluorescent Studies

**Tissue preparation.** Blocks of kidney cortex (2–3 mm³) were harvested from anesthetized rats. The tissues were immediately immersed and fixed in 3% paraformaldehyde. After fixation, the tissues were immediately immersed in histological cassettes and dehydrated with ethanol series, infiltrated with low-melting (50°C) paraplast, and embedded in high-melting (56°C) paraplast. The blocks were sectioned (4 μm) using an automated microtome (Micron HM355; Fisher, Pittsburg, PA).

**Immunohistochemistry.** Fixed kidney tissue was deparaffinized in CitriSolv and rehydrated in ethanol and HEPES wash buffer (Fisher Bioreagents, Fairlawn, NJ; distilled water, 900 ml; NaCl, 4.10 g; HEPES, 7.14 g; and CaCl₂, 0.29 g, pH 7.4). Epitopes were retrieved (antigen retrieval) in citrate buffer for 25 min at 95°C with a steamer. Slides were then immediately transferred into a humidity chamber. Nonspecific binding sites were blocked (5% BSA, 5% serum) at room temperature for 4 h. After blocking, the first section was washed (3 x 15 min) with HEPES wash buffer and then mounted with Mowiol (first control level).

The second section was washed and incubated with 1:100 primary antibodies in 10-fold-diluted blocking agent, and third/fourth sections were washed and kept in the blocker. Over the course of 48 h, a fifth section was incubated with 1:100 goat gp91phox primary antibody (Santa Cruz, Santa Cruz, CA), and a sixth section was incubated with 1:100 mouse Rac1 antibody (Upstate Cell Signaling Solutions, Lake Placid, NY) in 10-fold-diluted blocker at room temperature. After 24 h, the slides were washed (3 x 15 min), and a section was mounted with Mowiol (second control level). The rest of the sections were incubated with 1:300 AlexaFluor rabbit anti- goat-647 in 10-fold-diluted blocker except the sixth section, which was stained with 1:300 AlexaFluor goat anti-mouse-647 (Molecular Probes, Eugene, OR). After 4 h, the slides were washed (3 x 15 min), mounted with Mowiol, and stored in light tight slide boxes at 4°C. The slides were then examined using a laser confocal scanning microscope (50X laser, 2.3 Iris, 56 gain, zoom 1, and 00 offset; Bio-Rad, Cambridge, MA); images were captured by use of Laser-Sharp software (Bio-Rad), and signal intensities were measured by MetaVue (Boyece Scientific, Gray Summit, MO).

### Western Blot Analysis

Kidney cortex tissue was homogenized using a glass-on-glass Dounce homogenizer in sucrose homogenization buffer [0.25 M sucrose, 0.5 mM EDTA, 50 mM HEPES, protease inhibitors (Complete, Roche Diagnostics), phosphate inhibitors (Cocktail-1 and -2, Sigma Chemical)] and centrifuged at 1,000 g to remove connective tissue. Protein concentrations were measured by OPA as above. Supernatants were analyzed under denaturing conditions with SDS-PAGE using a Mini-Protein 2 electrophoresis system (Bio-Rad, Hercules, CA). Protein (30 μg) was mixed with 5 μl of loading buffer and incubated at 92°C for 5 min. Samples were then loaded in wells of 4–15% precast gels (Bio-Rad) and run at 180 V for 35 min. Proteins were transferred onto a nitrocellulose membrane in a wet transfer system (Bio-Rad) at 30 V overnight. Blots were blocked with 3% nonfat dry milk in PBS for 4 h. Membranes were incubated with primary antibodies anti-gp91phox (rabbit polyclonal) at 1:250 (Upstate Cell Signaling, Lake Placid, NY) and anti-Rac1, clone 238A (mouse monoclonal), at 1:1,000 (Upstate Cell Signaling) in 3% nonfat dry milk in PBS overnight. Membranes were washed and incubated for 90 min with secondary antibody (1:5,000) in 3% nonfat dry milk in PBS, using anti-rabbit horseradish peroxidase (HRP)-linked antibody (H&L Cell Signaling) for gp91phox and HRP-conjugated goat anti-mouse antibody (H&L; Jackson Immuno Research Laboratories, Westgrove, PA) for Rac1. Blots were incubated with enhanced chemiluminescence for 1 min, and the protein bands were visualized by use of ChemiDoc XRS (Bio-Rad) and quantified by Quantity One 1-D analysis software (Bio-Rad). Equal loading of total proteins in each lane was checked by Amido black staining using a densitometer.

### Podocyte Transmission Electron Microscopy

Upon death, kidney cortex tissue was placed in primary fixative (2% glutaraldehyde, 2% paraformaldehyde in 0.1 M Na-cacodylate buffer, pH 7.35). Samples were processed through a microwave oven (Pelco 3440) for secondary fixation, acetone dehydration, and Epon-Spur's resin infiltration. Samples were rocked overnight and then embedded and polymerized at 60°C for 24 h. Thin sections (85 nm) were collected using LeicaUltaract UCT microtome and a 45-degree Diatome diamond knife. Samples were stained with 5% uranyl acetate (UA) and Sato's triple lead stain (Pb). A JOEL 1200-EX transmission electron microscope was used to view the samples.

Three glomeruli per rat were evaluated with five 10k and 60k images per glomeruli. TEM images were analyzed using ImageJ (public domain, NIH), and analysis was adapted from previous work (21, 32); 10k images were used to analyze the number of slit pores per 1 μm of glomerular basement membrane. Straight basement membrane with uniform thickness and upright podocyte foot process was used to rule out bias sample cuts. One micrometer was calibrated.
Table 1. Experimental parameters between S-D and Ren2 rats

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<th>S-D</th>
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<tr>
<td>Weight, g</td>
<td>253±6</td>
<td>357±20</td>
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<td>162±10</td>
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<tr>
<td>1-h OGTT insulin, μU/mL</td>
<td>169±22</td>
<td>224±24</td>
<td>146±174</td>
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<tr>
<td>1-h Insulin-glucose ratio</td>
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<td>1.46±0.17</td>
<td>0.94±0.12‡</td>
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Values are means ± SE. S-D, Sprague-Dawley rats; Ren2, TG(mRen2)27 transgenic rats; Ren2-V, Ren2 rats treated with valsartan; 1-h OGTT, oral glucose tolerance test values 1 h after glucose feeding by gavage (1 g/kg body wt). *P < 0.05, S-D compared with Ren2. †P < 0.05, Ren2-V compared with Ren2. ‡P < 0.05, Ren2-V compared with S-D.

**Results**

**Blood Pressure and Albuminuria**

SBP were higher in Ren2 (200 ± 2 mmHg) than in S-D (120 ± 3 mmHg) rats (Table 1), but were significantly lower following valsartan treatment (Ren2-V, 140 ± 2 mmHg) compared with Ren2 (P < 0.05). Similarly, albumin-to-creatinine ratio (Fig. 1A) was higher in Ren2 (0.43 ± 0.36 mg/mg) than in S-D (0.08 ± 0.05 mg/mg, P < 0.05) rats, while albuminuria was decreased in Ren2-V (0.04 ± 0.03 mg/mg, P < 0.05) compared with Ren2 rats. Insulin resistance was also improved by valsartan treatment, as evidenced by significantly lower insulin and insulin-to-glucose ratio in Ren2-V compared with Ren2 (Table 1).

**Oxidative Stress**

MDA levels were elevated in Ren2 kidney tissue (0.49 ± 0.14 μg MDA/mg protein) compared with S-D (0.05 ± 0.01 heated at 50°C for 2 min and at 95°C for 10 min followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. All reactions were performed in triplicate, and 18S ribosomal RNA, amplified using the TaqMan Ribosomal RNA Control Kit (Applied Biosystems), served as an internal control. Results were quantified as C_t values, where C_t is the threshold cycle of PCR at which amplified product was first detected, and defined as relative gene expression (the ratio of target to control).

**Statistical Analysis**

Statistical analyses were performed with SPSS 13.0 (SPSS, Chicago, IL). We tested data for normality (Kolmogorov-Smirnov and Shapiro-Wilk) and homogeneity of variance (Levene) before conducting a two-way ANOVA (strain and treatment) with Bonferroni multiple comparisons test for each variable. In instances of significant interaction, data were further analyzed via one-way ANOVA with Bonferroni multiple comparisons. Data from filtration barrier measurements were then plotted against final systolic blood pressure, albuminuria, and MDA and then natural log (ln) transformed as appropriate for linear regression analysis. Pearson correlation coefficients were tabulated in SPSS. All values are expressed as means ± SE. P < 0.05 was considered statistically significant.
μg MDA/mg protein) but lower for Ren2-V (0.10 ± 0.01 μg MDA/mg protein) than for Ren2 (Fig. 1B). In addition, slit pore diameter was inversely associated with tissue MDA levels ($r^2 = 0.67$) in Ren2 and Ren2-V rats (Fig. 1D).

**Ultrastructural Analysis**

TEM images were used to evaluate four criteria for filtration barrier integrity: number of slit pores per 100 μm, basement membrane thickness (nm), podocyte foot process base width (nm), and slit pore diameter (nm) (Fig. 2). Significant changes in all four variables were observed when comparing Ren2 with S-D glomeruli, and these changes were attenuated with AT1R blockade in Ren2-V rats (Fig. 3).

There were fewer slit pores in Ren2 (394 ± 7 slit pores/100 μm, $P < 0.01$) than in S-D (486 ± 8 slit pores/100 μm) glomeruli, which was attenuated by AT1R blockade in Ren2-V rats (447 ± 12 slit pores/100 μm, $P < 0.01$) (Fig. 3). Similarly, slit pore diameter was less in Ren2 (25 ± 1 nm) glomeruli than in S-D (37 ± 2 nm, $P < 0.01$) (Fig. 3). Slit pore diameter also improved in Ren2-V (32 ± 2 nm) compared with Ren2 ($P < 0.01$). Basement membrane thickness was substantially greater for Ren2 (102 ± 5 nm) than for S-D (88 ± 4 nm) glomeruli ($P < 0.05$). This increase was eliminated in Ren2 after AT1R blockade (86 ± 2 nm) (Fig. 3). Podocyte foot process base width in Ren2 (219 ± 10 nm) was greater than for S-D (165 ± 6 nm, $P < 0.01$), while lower values were observed for Ren2-V Rat.

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**Fig. 2.** Images demonstrate podocyte and filtration barrier changes under transmission electron microscopy (TEM) in S-D rats. A: measurement of no. of slit pores per 100 μm at 10k (A). B: slit pore diameter (A), basement membrane width (B), and foot process base width (C) at 60k. C: podocyte foot process alignment and slit pores along a linear segment of basement membrane in Ren2-V. D: podocyte disruption and effacement in Ren2.

**Fig. 3.** Indexes of podocyte and filtration barrier change using TEM. A: measurement of no. of slit pores (SP) per 100 μm. B: basement membrane (BM) thickness (nm). C: slit pore diameter (nm). D: foot process base width (nm). *Significant difference between Ren2 and S-D and #significant difference between Ren2 and Ren2-V ($P < 0.05$).
kidneys (24/1 H11006 P Ren2 (tensinogen following AT1R blockade in Ren2-V. There was no expected increases in mRNA transcripts for renin and angio
ACE2, neprilysin, and the RAS in the kidney including renin, angiotensinogen, ACE, Tissue mRNA for RAS (0.83 and 0.18/1 H11006/1 H11006 (192
oxidase subunits gp91phox and Rac1. Fig. 4.

A: immunohistochemistry images of kidneys stained for NADPH oxidase subunits gp91phox and Rac1. B: measures of gray-scale intensity from glomeruli stained for gp91phox and Rac1. C: Western blot analysis for Rac1. D: Western blot analysis for gp91phox. PC, leukocyte positive control.

(192 ± 7 nm) compared with Ren2 (P < 0.05) (Fig. 4). Interestingly, slit pore number and slit pore diameter in kidneys from Ren2 and Ren2-V rats were inversely associated with albumin-to-creatinine ratio by linear regression (r² = 0.98 and 0.69, respectively; Fig. 4).

With the use of kidney immunohistochemistry images (Fig. 4), greater gray-scale intensities were observed for NADPH oxidase subunits gp91phox and Rac1 in Ren2 (43 ± 3 and 124 ± 7, respectively) compared with S-D rats (30 ± 4 and 100 ± 9, respectively). Following valsartan treatment, gp91phox and Rac1 gray-scale values were reduced in Ren2-V kidneys (24 ± 2 and 84 ± 11, respectively) compared with Ren2 (P < 0.05). There were similar trends for gp91phox (Fig. 6C) and Rac1 (Fig. 6D) proteins relative to total protein in Ren2 (0.83 ± 0.04 and 0.19 ± 0.01, respectively) compared with S-D kidneys (0.59 ± 0.01 and 0.18 ± 0.05, respectively) when Western blot techniques were used (P > 0.05). Ren2-V gp91phox and Rac1 relative protein abundances (0.74 ± 0.06 and 0.18 ± 0.01, respectively) were also lower than for Ren2 (0.83 ± 0.01 and 0.19 ± 0.01, respectively) (P > 0.05).

Relative mRNA levels were determined for components of the RAS in the kidney including renin, angiotensinogen, ACE, ACE2, neprilysin, and the mas receptor (Fig. 5). There were expected increases in mRNA transcripts for renin and angiotensinogen following AT1R blockade in Ren2-V. There was no difference (P > 0.05) between S-D, Ren2, and Ren2-V for kidney ACE mRNA. Neprilysin and ACE2 mRNAs were significantly greater in Ren2-V kidneys than in Ren2 (P < 0.05), but there was no difference in neprilysin or ACE2 between S-D and either Ren2 or Ren2-V (P > 0.05). Kidney mas receptor mRNA transcripts were more abundant (P < 0.05) in both Ren2 and Ren2-V compared with S-D but lower in Ren2-V compared with Ren2.

**DISCUSSION**

This investigation addressed the impact of AT1R blockade on glomerular filtration barrier integrity and oxidative stress in relation to components of the RAS. Filtration barrier integrity was evaluated in a hypertensive rat model of elevated renal ANG II (5, 22) by determining structural changes by TEM and by quantifying albuminuria. This transgenic model manifests increased ANG II and AT1R levels as well as ANG II binding to receptors in glomerular mesangial cells (34). Indeed, increased glomerular ANG II has been shown to contribute to the development of reductions in glomerular capillary permselectivity and albuminuria (2, 11, 17, 19). Consistent with this notion, we observed that the development of albuminuria in Ren2 rats was accompanied by loss of integrity of the glomerular filtration barrier. Electron microscopy measurements demonstrated podocyte foot process effacement, loss of slit pore diaphragm integrity, and widening of the bases of the podocyte foot process. Structural changes were associated with increases in oxidative stress and NADPH oxidase subunit proteins in renal cortical tissue. These changes are in concert with prior observations of increased NADPH oxidase activity and reactive oxygen species (ROS) in other tissues in the Ren2 model (3, 27). Furthermore, both abnormalities, increased ROS and loss of glomerular filtration integrity (albuminuria), were prevented by treating 6- to 7-wk-old Ren2 rats with an AT1R blocker for 3 wk.

Development of albuminuria is a cardinal manifestation of glomerular injury and loss of filtration barrier and a pathophysiological factor in the progression of renal dysfunction (31). The podocyte is the most differentiated cell type within the glomerular complex (25). It is an integral component of the glomerular basement membrane and slit pore diaphragm, cru
cital for the maintenance of the glomerular filtration barrier (25). Podocyte injury leads to destabilization of the foot process/slit pore diaphragm complex with resultant loss of glomerular permselectivity, resulting in albuminuria (16). Although podocytes have properties of terminal differentiated epithelial cells, rat podocytes have cytoskeletal structural components reminiscent of vascular smooth muscle cells (VSMC) (26). Similar to VSMC, the integrity of the cytoskeleton is integral to normal health and function of the podocyte. Loss of this cytoskeletal integrity may destabilize and disrupt the filtration slit membrane structure, leading to broadening of the base of podocytes, disruption of the filtration slit structure, and subsequent podocyte foot process effacement and albuminuria. All of these changes were observed in the untreated Ren2 rats, which have increased expression of glomerular ANG II and AT₁R (34). ANG II regulates glomerular filtration, in part, by modulation of arteriolar tone and ultrafiltration coefficient K (18, 31, 33).

Another mechanism by which excess ANG II may damage the glomerular filtration barrier is through enhancement of NADPH oxidase activity and generation of ROS (16, 27, 31). In untreated Ren2 rats, there were increased levels of MDA, a marker for ROS. This increase in oxidative stress was associated with a trend for increased membrane Rac1 and gp91phox subunits. Membrane localization and phosphorylation of these subunits are necessary for activation of the NADPH oxidase complex (13, 27, 33). Therefore, our data suggest that in vivo treatment with the AT₁R blocker valsartan decreases NADPH oxidase subunit activation. Furthermore, documentation of filtration barrier abnormalities in kidneys for Ren2 rats provides evidence that ANG II-mediated oxidative stress leads to podocyte disruption and loss of filtration barrier integrity.

In dissecting the impact of AT₁R blockade on renal function, it is important to consider the opposing effects that ANG-(1–7) has in countering the effects of excess ANG II and AT₁ signaling on glomerulotubular balance (9). Hypertension in Ren2 rats was associated with a reduced expression of kidney renin mRNA and increased kidney AT₁R mRNA. Administration of valsartan induced significant increases in kidney renin, angiotensinogen, ACE2, and nephrilysin mRNAs, findings that support a role for increased ANG-(1–7) activity as a mechanism contributing to the renoprotective effects of AT₁R blockade. In previous studies, we reported increased ACE2 activity in normotensive rats treated with either an ACE inhibitor or an AT₁R blocker (8). The observation that in vivo treatment with AT₁R blockade was associated with increased ACE2 and nephrilysin steady-state RNA levels is compatible with this interpretation, since ACE2 metabolizes ANG II into ANG-(1–7), while nephrilysin is the primary ANG-(1–7)-forming enzyme from ANG I (7). The shift in balance in the renal RAS may contribute to less oxidative stress, glomerular structural changes, and lesserened albuminuria following AT₁R blockade.

In conclusion, this investigation demonstrates that proteinuria in an insulin-resistant, hypertensive rodent model of tissue angiotensin overexpression is associated with enhanced generation of ROS and loss of glomerular filtration barrier integrity. In vivo treatment with an AT₁R blocker substantially reduced oxidative stress and glomerular filtration injury in association with improvements in albuminuria, blood pressure, and insulin sensitivity. These data add further credence to the notion that angiotensin stimulation of NADPH oxidase may be a common pathophysiological instigator of all of these abnormalities. These data also suggest that AT₁R blockade maintains glomerular filtration integrity, in part, by shifting the RAS balance. This work should provide the framework for additional research evaluating the impact of the RAS on glomerular injury.

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