Differential Effects of Angiotensin II Type-1 Receptor Antisense Oligonucleotides on Renal Function in Spontaneously Hypertensive Rats

Minoru Yoneda, Hironobu Sanada, Junichi Yatabe, Sanae Midorikawa, Shigeatsu Hashimoto, Midori Sasaki, Tetsuo Katoh, Tsuyoshi Watanabe, Peter M. Andrews, Pedro A. Jose, Robin A. Felder

Abstract—The effect of selectively decreasing renal angiotensin II type 1 (AT1) receptor expression on renal function and blood pressure has not been determined. Therefore, we studied the consequences of selective renal inhibition of AT1 receptor expression in normotensive Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) in vivo. Vehicle, AT1 receptor antisense oligodeoxynucleotides (AS-ODN), or scrambled oligodeoxynucleotides were infused chronically into the cortex of the remaining kidney of conscious, uninephrectomized WKY and SHR on a 4% NaCl intake. Basal renal cortical membrane AT1 receptor protein was greater in SHR than in WKY. In WKY and SHR, AS-ODN decreased renal but not cardiac AT1 receptors. AT1 receptor AS-ODN treatment increased plasma renin activity to a greater extent in WKY than in SHR. However, plasma angiotensin II and aldosterone were increased by AS-ODN to a similar degree in both rat strains. In SHR, sodium excretion was increased and sodium balance was decreased by AS-ODN but had only a transient ameliorating effect on blood pressure. Urinary protein and glomerular sclerosis were markedly reduced by AS-ODN–treated SHR. In WKY, AS-ODN had no effect on sodium excretion, blood pressure, or renal histology but also modestly decreased proteinuria. The major consequence of decreasing renal AT1 receptor protein in the SHR is a decrease in proteinuria, probably as a result of the amelioration in glomerular pathology but independent of systemic blood pressure and circulating angiotensin II levels. (Hypertension. 2005;46:58-65.)

Key Words: receptors, angiotensin II ■ rats ■ kidney ■ hypertension, essential ■ proteinuria

Genetic manipulations that increase the activity of the renin-angiotensin system (RAS) systemically1–5 or in specific organs (eg, brain and kidney) elevate blood pressure.6,7 For example, overexpression of angiotensinogen and renin in renal proximal tubules6 or in neurons and glia cells in brain7 increased blood pressure without increasing circulating renin levels. Systemic deletion or silencing of genes that regulate the RAS also decreases blood pressure. Thus, disrupting or silencing angiotensinogen4 and angiotensin-converting enzyme 1 genes8,9 or the angiotensin II type 1A receptor (AT1AR) gene10,11 in mice or rats decreases blood pressure. Decreasing the expression of liver angiotensinogen also decreases blood pressure in mice.12 More recently, Crowley et al reported that nephrectomized AT1A−/− mice with 1 transplanted AT1A+/+ kidney had higher blood pressures than those observed in unmanipulated AT1A−/− mice or AT1A−/− mice with a transplanted AT1A−/− kidney but lower than the blood pressures in unmanipulated AT1A+/+ or AT1A+/− mice with a transplanted AT1A+/+ kidney.13 These studies showed the importance of renal and extrarenal factors in the regulation of blood pressure. However, there are no reports of the renal functional and blood pressure consequences of silencing the AT1AR selectively in the kidney of rats with spontaneous hypertension. To test the hypothesis that selective renal inhibition of AT1AR expression differentially regulates renal function and blood pressure in normotensive and hypertensive rats, we determined the role of the intrarenal RAS on renal function by the chronic intrarenal cortical infusion of AT1AR oligodeoxynucleotides (ODN) in normotensive Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR).

Materials and Methods

Animals

Forty 3-week-old male WKY and SHR (Japan SLC Inc.; Sendai, Japan) were fed 0.28% NaCl chow (CLEA Japan) and tap water. At 4 weeks of age, the diet was changed to 4% NaCl chow. The high sodium intake was instituted to suppress the RAS but maintain a high level of AT1A expression in the kidney; high sodium intake increases renal but decreases extrarenal AT1A-R expression.14 All procedures...

Received January 26, 2005; first decision February 25, 2005; revision accepted May 16, 2005.
From the Fukushima Medical University School of Medicine (M.Y., H.S., J.Y., S.M., S.H., M.S., T.K., T.W.), Japan; Georgetown University Medical Center (P.M.A., P.A.J.), Washington, DC; and University of Virginia Health Sciences Center (R.A.F.), Charlottesville.
Correspondence to Pedro A. Jose, MD, PhD, Department of Pediatrics, PHC-2, Georgetown University Medical Center, 3800 Reservoir Rd NW, Washington, DC 20007. E-mail pjose01@georgetown.edu

Hypertension is available at http://www.hypertensionaha.org

DOI: 10.1161/01.HYP.0000171587.44736.ba
were approved by the Fukushima Medical University School of Medicine animal committee.

**Design and Synthesis of AT1R ODN**

Purified phosphorothioate-modified rat AT1R AS-ODN (5'-AGAGTTAAGGGCCAT-3') and scrambled ODN (SC-ODN; 5'-CCCTTTGAAAGTTCC-3') were synthesized at the DNA Synthesis Core Laboratory of the Bowman Gray School of Medicine. The AS-ODN sequence is 100% homologous to rat AT1R (X62295) and 93% homologous to rat AT1R (AH004552) and unique to the AT1Rs. AS-ODN but not SC-ODN causes a 50% decrease in AT1R protein, measured by radioligand binding in rats. 15

**Uninephrectomy and Renal Cortical Interstitial Catheter Implantation**

Three-week-old rats were allowed to acclimatize for 1 week. At 4 weeks of age, under intraperitoneal pentobarbital anesthesia (50 mg/kg), the right kidney was removed, and a catheter (8-mm PE 10 tube connected to medical teflon microtubing (BB311-30, 30 gauge; Scientific Commodities, Inc) by Bipax epoxy resin glue was implanted 3- to 4-mm deep into the lower pole of the remaining left kidney. 17 Uninephrectomy was performed to obviate the interstitial infusion of the vehicle and ODN to both kidneys, which would necessitate extra surgery. Unequal inhibition of AT1R expression in the 2 kidneys could also confound the interpretation of the results. An osmotic mini-pump (1 μL per hour; Alzet Corporation) was positioned in the space occupied previously by the right kidney for the continuous interstitial infusion of lactated Ringer’s solution. After 7 days (5 weeks of age), rats were reanesthetized and the implanted osmotic mini-pump replaced with another that infused AT1R AS-ODN, SC-ODN (50 nmol/L per day), or vehicle (lactated Ringer’s solution) at 0.2 μL per hour.

**Effect of AT1 ODNs on Urinary Sodium and Potassium Excretion and Systolic Blood Pressure**

Urine was collected for 24 hours twice per week. Sodium, potassium, and creatinine were measured with automated methods (Hitachi 7600-120). Unanesthetized systolic blood pressures were measured twice per week by the tail-cuff method (blood pressure analyzer model BP-98A; Softron).

**AT1R in the Kidney**

After 4 weeks of renal cortical infusion, rats were anesthetized, and blood was collected through a cardiac catheter and perfused with 50 mL of lactated Ringer’s solution. The kidney and heart were quickly removed, weighed, flash-frozen in liquid nitrogen, and stored at -70°C. In some rats, kidneys were fixed with Histochoice and cryoprotected with 30% sucrose for the fluorescence microscopic localization of rhodamine-conjugated AS-ODN, SC-ODN, or vehicle.

**Immunoblotting**

Renal cortical (upper pole, left kidney) and cardiac ventricular proteins were immunoblotted with rabbit anti-human AT1R antibody (sc-1173; Santa Cruz Biotechnology) as reported. 17 The immunizing peptides QDDCPKAGRH correspond to amino acids 15 to 24 of the AT1R 17 The specificity of this AT1R antibody has been reported. Nonetheless, we also performed preadsorption studies with the immunizing peptide (sc-1173P; Santa Cruz Biotechnology); the AT1R antibodies were incubated overnight at 4°C with a 10-fold molar excess of the peptide. We found 2 bands between 45 and 48 kDa (visualized by enhanced chemiluminescence [ECL] reagents; Amer sham Corp); the latter band not blocked by the immunizing peptide was considered nonspecific (data not shown). Indeed, the AT1R AS-ODN inhibited the expression of the 45-kDa band but not the 48-kDa band (Figure 1). Bands were quantified by densitometry (QuantiScan). The amount of protein transferred onto the membranes was verified by immunoblotting for β-actin.

**Histochemistry**

In additional experiments, kidneys were fixed overnight in 10% buffered formalin, dehydrated, and imbedded in paraffin. Then 1- to 3-μm sections were stained with periodic acid-Schiff (PAS) reagent, viewed, photographed, and evaluated blindly by 3 independent investigators. The degree of glomerulosclerosis was evaluated using a semiquantitative score (glomerulosclerosis index [GSI]). Sclerosis was defined as collapse or obliteration of the glomerular capillary tuft associated with increased hyaline matrix. In each single section of kidney, all glomeruli (ie, superficial and juxtaglomerular) were assessed for glomerulosclerosis. The severity of sclerosis for each glomerulus was graded from 0 to 4+. No lesions were graded as 0, lesions of 10% or less of the glomerulus were graded as 1, lesions of up to 25% of the glomerulus were graded as 2, lesions of up to 50% of the glomerulus were graded as 3, and lesions of up to 100% of the glomerulus were graded as 4.

**Renin-Angiotensin System**

Blood was centrifuged at 3000 g for 15 minutes at 4°C, and the plasma was stored at -80°C until analysis. Plasma renin activity (PRA), angiotensin II, and aldosterone concentrations were measured by radioimmunoassay.

**Statistical Analysis**

Data are expressed as mean ± SE. Comparisons within and among groups were made by repeated-measures or factorial ANOVA, respec-
tively, followed by Holm–Sidak or Duncan’s test. A value of \( P<0.05 \) was considered significant.

**Results**

There were no differences in food and water intake before (4 weeks old), during, or at the end (9 weeks old) of the study (Tables 1 and 2). There were also no differences in body weight at the beginning of the study. By the end of the study, SHR weighed less than WKY, but their weights were not affected by AT1R ODIN treatment. The heart weight (percentage body weight) was greater in SHR than in WKY and greater in AT1R AS-ODN–treated than in vehicle- or AT1R SC-ODN–treated SHR (Table 2). Blood pressures were higher in SHR than in WKY at the beginning and end of the study. Kidney weights were initially similar among the groups and remained similar in those not treated with AT1R AS-ODN; AS-ODN treatment decreased kidney weights to the same degree in WKY and SHR. Serum creatinine was similar in all the groups and unaffected by ODIN treatment (Table 2).

**Angiotensin II Type 1 Receptors**

AT1R protein (corrected for \( \beta \)-actin) in renal cortical membranes was greater in SHR (1.54±0.19; \( n=3 \)) than in WKY (0.92±0.05; \( n=3 \); \( t \) test). ODINs did not affect cardiac AT1R protein. However, AT1R AS-ODN but not AT1R SC-ODN decreased AT1R protein in renal cortical membranes of WKY and SHR (Figure 1). These effects of AS-ODN on AT1R expression support the specificity of the AT1R antibody and the AT1R AS-ODN used in these experiments. The magnitude of the decrease in AT1R expression caused by the AT1R AS-ODN is similar to those reported by Li et al, who used the same ODINs but measured AT1R protein by radioligand binding.15

**TABLE 1. Characteristics of 4-Week-Old WKY and SHR Before Unilateral Nephrectomy and Insertion of an Intracortical Catheter Into the Remaining Kidney**

<table>
<thead>
<tr>
<th>Variables</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle (n=5)</td>
<td>SC-ODN (n=6)</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>81.8±7.0</td>
<td>84.2±5.6</td>
</tr>
<tr>
<td>Kidney weight (% body weight)</td>
<td>0.62±0.04</td>
<td>0.59±0.04</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>89.5±0.6</td>
<td>88.7±0.5</td>
</tr>
<tr>
<td>Water intake (ml/day)</td>
<td>14.2±0.6</td>
<td>13.3±0.9</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>10.0±0.3</td>
<td>9.5±0.6</td>
</tr>
<tr>
<td>Urine output (ml/day)</td>
<td>2.5±0.3</td>
<td>3.2±0.4</td>
</tr>
<tr>
<td>Na+ excretion (mmol/L per day)</td>
<td>0.42±0.03</td>
<td>0.46±0.05</td>
</tr>
<tr>
<td>K+ excretion (mmol/L per day)</td>
<td>1.06±0.08</td>
<td>1.01±0.11</td>
</tr>
<tr>
<td>Proteinuria (mg/day)</td>
<td>0.31±0.07</td>
<td>0.55±0.16</td>
</tr>
</tbody>
</table>

\( P<0.05 \); *vs WKY; †vs others within their respective groups; ‡vs all others; §vs SHR; ANOVA; Holm–Sidak test or Duncan’s test.

**TABLE 2. Characteristics of WKY and SHR (9 weeks) at the End of the Study (9 weeks)**

<table>
<thead>
<tr>
<th>Variables</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle (n=8)</td>
<td>SC-ODN (n=8)</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>247±3</td>
<td>242±3</td>
</tr>
<tr>
<td>Remaining kidney weight (% body weight)</td>
<td>0.72±0.01</td>
<td>0.73±0.01</td>
</tr>
<tr>
<td>Heart weight (% body weight)</td>
<td>0.48±0.01</td>
<td>0.47±0.02</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>140±1</td>
<td>146±2</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.21±0.01</td>
<td>0.20±0.01</td>
</tr>
<tr>
<td>PRA (ng/mL per hour)</td>
<td>0.22±0.08</td>
<td>0.20±0.04</td>
</tr>
<tr>
<td>Plasma aldosterone concentration (mg/dL)</td>
<td>35.1±3.6</td>
<td>36.7±2.6</td>
</tr>
<tr>
<td>Angiotensin II (pg/mL)</td>
<td>4.7±0.5§</td>
<td>5.5±0.6§</td>
</tr>
<tr>
<td>Water intake (ml/day)</td>
<td>49.8±1.6</td>
<td>53.7±2.2</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>16.8±0.5</td>
<td>18.2±0.4</td>
</tr>
<tr>
<td>Urine output (ml/day)</td>
<td>38.9±2.5</td>
<td>41.5±1.5</td>
</tr>
<tr>
<td>Na+ excretion (mg/day)</td>
<td>10.6±0.4</td>
<td>11.5±0.6</td>
</tr>
<tr>
<td>Na+ balance (mmol/L per day)</td>
<td>1.58±0.25</td>
<td>1.48±0.47</td>
</tr>
<tr>
<td>K+ excretion (mmol/L per day)</td>
<td>3.03±0.04</td>
<td>3.02±0.21</td>
</tr>
<tr>
<td>Proteinuria (mg/day)</td>
<td>25.85±4.03</td>
<td>31.5±4.5</td>
</tr>
</tbody>
</table>
PRA, Angiotensin II, and Aldosterone Levels

PRA, angiotensin II, and plasma aldosterone concentrations were higher in SHR than in WKY. Their levels were increased by AT,R AS-ODN treatment in both rat strains but continued to be higher in SHR than in WKY (Table 2). Although the fold increase in PRA with AT,R AS-ODN was greater in WKY (6.90 ± 0.66) than in SHR (2.05 ± 0.20; \( P < 0.05 \)), the fold increase in angiotensin II levels with AT,R AS-ODN was similar (WKY 3.67 ± 0.43; SHR 3.53 ± 0.51). The increase in PRA and angiotensin II levels with AT,R AS-ODN treatment was probably caused by interruption of the renin and angiotensin II negative feedback.\(^1^8\)

Blockade of AT,Rs should result in a decrease in aldosterone secretion. However, urinary and plasma aldosterone levels were not different between AT,\(_1\)A\(^{-/-}\) and AT,\(_1\)A\(^{+/+}\) mice fed a high-sodium diet. Urinary and plasma aldosterone increased to similar levels in AT,\(_1\)A\(^{-/-}\) and AT,\(_1\)A\(^{+/+}\) mice fed a low-sodium diet.\(^1^9\) The infusion of a nonselective AT,\(_1\)A/\(_1\)B antagonist, CV-11974, in AT,\(_1\)A\(^{-/-}\) mice decreased basal plasma aldosterone levels and prevented the stimulatory effect of angiotensin II on aldosterone,\(^2^0\) indicating the importance of both AT,R subtypes in the regulation of aldosterone secretion. In our studies, AT,R AS-ODN increased aldosterone concentration to a similar degree in chronically salt-loaded WKY (1.83 ± 0.29) and SHR (2.7 ± 0.37), but the absolute levels remained higher in SHR than in WKY (Table 2). The increase in aldosterone concentration with AT,R AS-ODN treatment probably occurred as a result of the stimulation of adrenal cortical AT,Rs by the increased circulating levels of angiotensin II. This result provides additional evidence that the decrease in renal AT,R protein caused by AT,R AS-ODN was confined to the kidney.

Blood Pressure

The blood pressure increased with age to a greater extent in SHR than in WKY (Figure 2; Table 2). The renal cortical infusion of AT,R AS-ODN had no effect on blood pressure in WKY, whereas it transiently lowered blood pressure in SHR at 7 weeks of age relative to the vehicle- and SC-ODN–treated SHR. At 8.5 weeks of age, AT,R AS-ODN–treated SHR had higher blood pressure than their vehicle- or SC-ODN–treated counterparts (Figure 2).

Urine Flow and Sodium and Potassium Excretion

Baseline urine, sodium, and potassium outputs were similar among the groups (Table 1). With age, urine, sodium (Figure 3), and potassium outputs increased in all rats. Urine and potassium outputs were not affected by ODN treatment in either rat strain. AT,R AS-ODN treatment had no effect on sodium excretion or sodium balance in WKY (Table 2).

In SHR, at 6 weeks of age and 1 week after the start of the intrarenal cortical infusion of AT,R AS-ODN, sodium excretion increased and approached those observed in WKY (Figure 3; Table 2). At 7.5 weeks of age, sodium balance was
greater in SHR than in WKY. In AT1R AS-ODN–treated SHR, sodium balance was less (1.92 ± 0.50 mEq per day) than those observed with vehicle (4.25 ± 0.66 mEq per day) or AT1R SC-ODN treatment (3.75 ± 0.65 mEq per day). This was reflected in body weights; AT1R AS-ODN–treated SHR (166 ± 6 g) weighed less than vehicle- (176 ± 7 g) or SC-ODN– (176 ± 9 g) treated SHR. The AT1R AS-ODN–induced increase in sodium excretion and decreased positive sodium balance in SHR persisted until the end of the study, but body weights eventually became similar (Table 2).

Urine Protein Excretion
Baseline urinary protein excretion was similar among the groups (Table 1). Protein excretion increased after 7 weeks of age in all the groups but to a greater extent in vehicle and AT1R SC-ODN–treated SHR than the other groups (Figure 4). AT1R AS-ODN treatment decreased protein excretion in WKY at 9 weeks of age. In SHR, AT1R AS-ODN treatment markedly reduced protein excretion and became similar to those observed in WKY (Table 2; Figure 4).

Histochemistry
There were no differences in the histology of the nephrectomized kidneys (5 weeks) of WKY and SHR (data not shown). At 9 weeks of age, the glomeruli in WKY were normal appearing and not affected by ODN treatment (Figure 5a through 5c). In contrast, at 9 weeks of age in SHR, glomerulosclerosis was evident in vehicle- (Figure 5d) and AT1R SC-ODN–treated SHR (Figure 5e). However, AT1 AS-ODN–treated SHR had marked improvement in glomerular histopathology (Figure 5f). The improvement in GSI in AT1 AS-ODN–treated SHR is shown in Figure 6.

Discussion
Several studies have suggested that the long-term regulation of blood pressure rests on renal and nonrenal mechanisms.21–24 One of the important mechanisms of blood pressure control is the RAS, which also regulates blood pressure by renal and nonrenal mechanisms.25,26 Systemic disruption or silencing of genes that regulate the RAS, such as angiotensinogen, angiotensin-converting enzyme I, and AT1A/R in mice and rats, decreases blood pressure.4–12 However, the contribution of renal and nonrenal mechanisms could not be determined from these previous reports. To determine the contribution of renal AT1Rs in the regulation of blood pressure, Crowley et al studied the effect of transplanting kidneys from AT1A/–/– mice into bilaterally nephrectomized wild-type nontransgenic mice, and vice versa.13 These investigators estimated that basal blood pressure control resides in AT1Rs inside and outside the kidney.

In uninephrectomized rats fed 4% NaCl, intrarenal AT1R AS-ODN decreased renal AT1R protein expression. The inhibition of AT1R expression was selective to the kidney because AT1R expression in the heart was not altered. The increase in circulating aldosterone with AT1R AS-ODN provided additional evidence that the decrease in AT1R protein caused by AT1R AS-ODN was confined to the kidney. The fold increase in circulating angiotensin II levels was similar in WKY and SHR, although circulating angiotensin II levels were still 2-fold higher in SHR than in WKY. AT1R AS-ODN treatment had no effect on blood pressure in WKY and only a transient effect in SHR; the absolute blood pressure levels continued to be higher in SHR than in WKY. The lack of effect of inhibition of renal AT1R expression on blood pressure may have been caused by the elevation in the circulating levels of angiotensin II in the AS-ODN–treated groups. AT1R AS-ODN–treated WKY had plasma angiotensin II levels similar to those noted in vehicle- and AT1R SC-ODN–treated SHR. Nevertheless, AT1R AS-ODN–treated WKY continued to have lower blood pressures than vehicle or ODN-treated SHR, suggesting that mechanisms in addition to angiotensin II levels (eg, increased vascular reactivity to angiotensin II) are important in the high blood pressure of SHR.

The ability of the kidney to regulate sodium transport is important in the long-term regulation of blood pressure.25,26 We found that decreased renal AT1R protein was associated with an increase in sodium excretion and a decrease in positive sodium balance in AT1R AS-ODN–treated SHR but not WKY. The sodium balance in our studies was calculated without taking into account fecal sodium excretion. SHR have been reported to have increased intestinal sodium transport,27,28 although fecal sodium balance was not different between WKY and SHR.29 The decrease in positive sodium balance in AS-ODN–treated SHR was associated initially
with lesser weight gain. The increase in sodium excretion engendered by the decrease in renal AT\(_1\)R protein in SHR in the face of increased circulating aldosterone indicates that AT\(_1\)Rs can regulate sodium transport, independent of aldosterone (eg, proximal tubule; see Perspectives). It is possible that the blood pressure of the AT\(_1\)R AS-ODN–treated SHR was not different from the vehicle- and AT\(_1\)R SC-ODN–treated SHR because 2 counteracting effects (increased angiotensin II levels and increased sodium excretion) cancelled each other.

One of the major novel observations in this study is the decrease in urinary protein excretion after the decrease in renal AT\(_1\)R protein expression in WKY and SHR, despite the absence of any decrease in blood pressure. Angiotensin-converting enzyme inhibitors, alone or in combination with AT\(_1\)R blockers, have been shown to decrease urine protein excretion, even in normotensive subjects and independent of blood pressure.\(^2,3^0\) In diabetic subjects whose aldosterone levels increased during angiotensin-converting enzyme inhibition, the addition of an aldosterone blocker decreased urinary protein even further, without any changes in blood pressure.\(^3^1\) In our studies, the decrease in urinary protein excretion by AT\(_1\)R AS-ODN occurred in WKY and SHR, but the decrease in protein excretion was greater in SHR such that it was no longer different from that seen in AS-ODN–treated WKY. The decrease in proteinuria in WKY and SHR did not correlate with blood pressure. Therefore, the magnitude of the decrease in proteinuria in salt-loaded SHR, which continued to be hypertensive, supports the notion that the antiproteinuric effect of AT\(_1\)R blockade could not be explained by reduction in blood pressure.\(^3^2\) Given the limitation of the current studies and the tail-cuff monitoring of blood pressures, our report may not provide a definite answer to the question as to whether the renoprotective effect of AT\(_1\)R blockade is or is not dependent on blood pressure reduction.\(^3^2,3^3\)

The other major novel observation in our study is the decrease in urinary protein excretion after the decrease in renal AT\(_1\)R protein expression in WKY and SHR despite the elevated circulating aldosterone concentrations. Aldosterone has been shown to be important in the pathogenesis of proteinuria, and aldosterone blockade decreases urinary protein excretion.\(^3^2–3^5\) Plasma aldosterone concentration was markedly elevated by AT\(_1\)R AS-ODN treatment, yet the urinary protein excretion decreased in WKY and SHR. The marked decrease in proteinuria afforded by AT\(_1\)R AS-ODN treatment in SHR was associated with normalization in glomerular histology. These studies indicate that the antiproteinuric effect of AT\(_1\)R suppression is independent of aldo-
The selective decrease in renal AT1 expression increases SHR, probably because of increased angiotensin II levels. 

Several studies have shown that angiotensin II, via AT1Rs, can have effects on renal function independent of aldosterone. The AT1R-dependent but aldosterone-independent alteration in sodium excretion may occur in the proximal tubule,\textsuperscript{26,36,37} where aldosterone receptors are not expressed.\textsuperscript{38,39} Although aldosterone may have nongenomic effects in human proximal tubule cells,\textsuperscript{40} such effects have not been described in rodents.

Perspectives
Several studies have shown that angiotensin II, via AT1Rs, can have effects on renal function independent of aldosterone. The AT1R-dependent but aldosterone-independent alteration in sodium excretion may occur in the proximal tubule,\textsuperscript{26,36,37} where aldosterone receptors are not expressed.\textsuperscript{38,39} Although aldosterone may have nongenomic effects in human proximal tubule cells,\textsuperscript{40} such effects have not been described in rodents.

References


Differential Effects of Angiotensin II Type-1 Receptor Antisense Oligonucleotides on Renal Function in Spontaneously Hypertensive Rats
Minoru Yoneda, Hironobu Sanada, Junichi Yatabe, Sanae Midorikawa, Shigeatsu Hashimoto, Midori Sasaki, Tetsuo Katoh, Tsuyoshi Watanabe, Peter M. Andrews, Pedro A. Jose and Robin A. Felder

Hypertension. 2005;46:58-65; originally published online June 13, 2005;
doi: 10.1161/01.HYP.0000171587.44736.ba

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/46/1/58

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/