Angiotensin-Converting Enzyme Inhibition and AT_1 Receptor Blockade Modify the Pressure-Natriuresis Relationship by Additive Mechanisms in Rats with Human Renin and Angiotensinogen Genes

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Abstract. The intrarenal factors responsible for hypertension in double-transgenic rats (dTGR) harboring human renin and human angiotensinogen genes are unclear. The pressure-natriuresis and -diuresis relationships in response to chronic angiotensin-converting enzyme (ACE) inhibition and AT_1 receptor blockade were evaluated. Renal renin-angiotensin and nitric oxide (NO) system gene expression was also investigated. Six-week-old dTGR were treated for 3 wk with submaximal doses of cilazapril (10 mg/kg, orally) or losartan (10 mg/kg, orally) or with the drug combination. In untreated dTGR, pressure-natriuresis relationships were maximally shifted rightward by approximately 70 to 80 mmHg, and both renal blood flow (RBF) and GFR were markedly decreased. Submaximal cilazapril and losartan dosages both decreased systolic BP by 30 mmHg and shifted the pressure-natriuresis curves leftward by 25 to 30 mmHg. Cilazapril increased RBF and GFR to values observed in normotensive control animals but did not significantly affect fractional sodium excretion (FENa) or fractional water excretion (FEH_2O) curves. In contrast, losartan had no significant effect on RBF or GFR but shifted the FENa and FEH_2O curves leftward. The cilazapril and losartan combination completely normalized BP and shifted the pressure-natriuresis curves leftward more than did either drug alone. When cilazapril and losartan were administered at higher doses (30 mg/kg, orally), the two drugs equally shifted the pressure-natriuresis curves leftward, by 50 mmHg. Both drugs increased RBF and GFR, however, only losartan shifted FENa and FEH_2O curves leftward. Human and rat renin and angiotensinogen genes were downregulated in dTGR and were increased by losartan and cilazapril treatments, whereas no changes in the expression of rat ACE and AT_1A receptor genes were observed. Endothelial NO synthase expression was increased by cilazapril but not by losartan. Neither inducible NO synthase nor neural NO synthase gene expression was affected by drug treatments. Therefore, submaximal ACE inhibition enhanced sodium excretion mainly by increasing RBF and GFR, whereas submaximal AT_1 receptor blockade decreased tubular sodium and water reabsorption. The combination of the two drugs produced an additive effect. The ACE inhibitor effects may involve increased endothelial NO synthase expression, perhaps related to the inhibition of bradykinin degradation.

The renin-angiotensin system (RAS) is important in the regulation of BP and is a key determinant of renal sodium and water excretion. In rats, physiologic and pharmacologic experiments designed to explore the role of the RAS in hypertension are hampered by the species specificity of the renin-angiotensinogen interaction. The hypertension of double-transgenic rats (dTGR) with the human renin (hRen) and human angiotensinogen genes is dependent on the human RAS components (1). These rats offer a unique opportunity to study the human RAS and pharmacologically induced changes in the human RAS in an experimental animal model. dTGR are also suitable for studying the lifelong effects of RAS overexpression, whereas experimental protocols using chronic angiotensin II (AngII) infusion via osmotic minipumps are usually limited to experimental periods of 1 to 2 wk. dTGR develop severe hypertension and nephrosclerosis at the age of 8 to 10 wk because of the human components of the RAS (1). We showed earlier that at 6 wk of age, when the kidneys of dTGR were histologically unaffected, the pressure-natriuresis and -diuresis relationships were already shifted toward higher operating BP levels (2). The altered renal excretory function in dTGR seemed to be intrinsically localized to the kidney, because clamping of the
neurohumoral factors by renal denervation and infusions of aldosterone, 17-hydroxycorticosterone, norepinephrine, or vasopressin did not change the renal pressure-natriuresis/diuresis relationships in these animals (2). The RAS also exerts multiple effects inside the kidney. AngII decreases sodium and water excretion by decreasing renal blood flow (RBF) and GFR. In addition, AngII directly stimulates sodium reabsorption in the proximal and distal tubules (3–5). To more specifically evaluate the pathophysiologic renal mechanisms responsible for the altered pressure-natriuresis relationships in dTGR, as well as to observe the effects on gene and transgene expression, we directly blocked AT1 receptors or we inhibited the angiotensin-converting enzyme (ACE). We observed that ACE inhibition and AT1 receptor blockade operated by distinct additive mechanisms.

Materials and Methods

Animal Protocols

Protocol 1. Experiments were conducted in 43 male dTGR (175 to 250 g) bearing the hRen gene and human angiotensinogen gene and in 10 normotensive control rats bearing only the hRen gene (hRen rats). The rats were purchased from Biological Research Laboratories (Fullsindorf, Switzerland). The procedures and experimental protocols were approved by the local Council on Animal Care, whose standards correspond to those of the American Physiological Society. The rats were allowed free access to standard rat chow (0.3% sodium; SSNIFF Spezialitäten, Soest, Germany) and drinking water.

In a preliminary study, we treated 6-wk-old dTGR (n = 6 in each group) with a single oral dose of cilazapril or losartan (10 mg/kg; 4 h before kidney function testing) and found no effects on pressure-natriuresis/diuresis curves, RBF, or GFR. These findings were in agreement with previous reports demonstrating no renal responses to AT1 blockade after acute treatment (6,7). We therefore decided to administer losartan and cilazapril chronically (10 mg/kg, by gavage, once each day for 3 wk). We divided the 6-wk-old, BP-, and body weight-matched dTGR into three treatment groups, which received either cilazapril (n = 10), losartan (n = 10), or vehicle (n = 15). Because neither cilazapril nor losartan completely normalized BP, another group of 6-wk-old dTGR (n = 8) were treated with the drug combination (both drugs at 10 mg/kg, by gavage) for 3 wk, in an additional study. Control dTGR and normotensive hRen rats received vehicle (5% gum arabicum). BP was measured by the tail-cuff method at the beginning of the study, for matching of the rats.

There were no differences among the treatment groups with respect to body weight gain during the follow-up period (ANOVA, P = 0.76). At the age of 9 wk, the rats were prepared as described previously (8). Because clamping of the neurohumoral factors by renal denervation and infusions of aldosterone, 17-hydroxycorticosterone, norepinephrine, or vasopressin did not change the renal pressure-natriuresis/diuresis relationships in dTGR in our previous study (2), only the protocol without neurohumoral clamping was used in this study. Briefly, the rats were anesthetized with an intramuscular injection of 35 mg/kg ketamine (Parke-Davis, Berlin, Germany) and an intraperitoneal injection of 65 mg/kg inactin (Research Biochemicals, Natick, MA). The rats were placed on a heated table to maintain their body temperatures at 37°C. Cannulae were inserted into the trachea to facilitate breathing, into the carotid and femoral arteries for measurement of renal perfusion pressure (RPP), into the jugular vein for compound infusion, and into the left ureter for urine collection. The right kidney was removed through a midline incision. An adjustable clamp was placed around the aorta above the left renal artery, and ligatures were loosely placed around the celiac and mesenteric arteries and around the aorta below the left renal artery, for later occlusion. During preparation, the rats were infused with a 0.9% saline solution containing 1% bovine albumin, at a rate of 50 µl/min per 100 g body wt. Insulin (20 mg/ml; Sigma) and para-aminohippurate (1.2 mg/ml; Merck Sharp & Dohme) were then included in the infusion for measurement of GFR and renal plasma flow, and the infusion rate was reduced to 33 µl/min per 100 g body wt.

After surgical preparation and an equilibration period of 60 min, RPP was lowered to 69 mmHg in hRen rats and 100 mmHg in untreated dTGR. After an additional 30-min equilibration period, urine flow, sodium excretion, GFR, and RBF were determined in two 20- to 30-min collection periods, depending on the magnitude of the urine flow. The supra-aortic occluder was then released, to increase RPP by approximately 30 mmHg. Again, urine and plasma samples were obtained after a 25- to 30-min equilibration period. RPP was finally increased to approximately 124 mmHg in hRen rats and 207 mmHg in untreated dTGR. After a 10-min equilibration period, urine and plasma samples were collected in two 5-min periods. In cilazapril- and losartan-treated dTGR, we were able to first decrease RPP to a level of 73 to 87 mmHg. We then increased RPP to 112 to 118 mmHg and finally to 152 to 175 mmHg. At these pressure levels, urine samples were collected in a manner similar to that used for untreated rats. In dTGR treated with the drug combination, RPP was first decreased to 70 mmHg; RPP was then increased to 102 mmHg and finally to 138 mmHg.

Protocol 2. Four-week-old BP- and body weight-matched dTGR were divided into three groups, which received either high-dose cilazapril (30 mg/kg, orally) (n = 5), high-dose losartan (30 mg/kg, orally) (n = 5), or vehicle (n = 7) for 3 wk. After surgical preparation as described above and a 60-min equilibration period, RPP in the dTGR control group was first lowered to 118 mmHg; RPP was then increased to 155 mmHg and finally to 199 mmHg. In cilazapril- and losartan-treated dTGR, RPP was first decreased to a level of 68 to 70 mmHg. We then increased RPP to 112 to 123 mmHg and finally to 144 to 149 mmHg. At these pressure levels, urine flow, sodium excretion, GFR, and RBF were determined as described for protocol 1.

General Procedures

Mean arterial BP was continuously measured throughout the experiment and was recorded on a computer system (TSE, Bad Homburg, Germany). Representative mean arterial BP values were determined before the start of pressure-natriuresis experiments (to characterize the BP levels of hRen rats and dTGR) and during each urine collection period, by averaging all values recorded during that period. Urine flow was determined gravimetrically. Insulin and p-aminohippurate concentrations of urine and plasma samples were determined according to methods outlined elsewhere (9,10). GFR was calculated as plasma insulin concentration ratio × urine flow. RBF was calculated as renal plasma flow/(1 − hematocrit). Urinary (FLM3, Radiometer, Copenhagen, Denmark) and plasma (Cobas Mira Plus; Roche, Basel, Switzerland) sodium concentrations were determined by flame photometry. Urine flow, sodium excretion, GFR, and RBF were normalized per gram of kidney wet weight. For estimation of left ventricular hypertrophy, the heart was excised, the great vessels, the atria, and the free wall of the right ventricle were dissected, and the left ventricular mass was measured. The left ventricular wet weight/body weight was calculated as an index of left ventricular hypertrophy.
**Tissue Preparation for Morphologic Analysis**

For conventional morphologic analysis, the kidneys were removed, cut sagittally, and fixed in 4% buffered paraformaldehyde, at room temperature. The tissue was dehydrated in graded alcohols and embedded in paraffin. Sections of 2- to 3-μm thickness were cut using a Leitz microtome (Leitz 1512; Leitz, Wetzlar, Germany). The sections were deparaffinized and rehydrated before being stained as outlined previously (11). The tissue was examined without knowledge of the identity of the rats.

**RNase Protection Assays and In Situ Hybridization**

For RNase protection assays, one-half of the right kidney was snap-frozen in isopentane (−35°C) and subjected to RNA isolation. The tissue was homogenized and the RNA was isolated by a standard lithium chloride/urea precipitation technique, as described in detail elsewhere (1). mRNA specific for human angiotensinogen, rat angiotensinogen, rat renin, hRen, and β-actin were identified by RNase protection assays with an Ambion RPA III kit (ITC Biotechnology, Austin, TX), according to the protocols provided by the manufacturer. For the detection of rat renin, hRen, and rat and human angiotensinogen mRNA, the probes and protocols described by Bohlender et al. (1) were used. For the detection of ACE and AT1A receptor mRNA, the probes described by Gross et al. (12) were used. The probes and procedures used for the detection of endothelial, inducible, and neural nitric oxide (NO) synthases were described in detail elsewhere (13).

**In situ hybridization** was performed for the localization of ACE and AT1A receptor genes in the kidney. The probes and the in situ hybridization procedures were described in detail previously (12,14).

**Statistical Analyses**

Data are presented as means ± SEM. Statistically significant differences in mean values were tested using two-way ANOVA for repeated measures and the Duncan multiple-range test. Linear regression lines were calculated using the least-squares method. Values of \( P < 0.05 \) were considered statistically significant.

**Results**

**Mortality Rates, BP, and Left Ventricular Hypertrophy with Protocols 1 and 2**

With protocol 1, the mortality rate for untreated dTGR during the experiment was 53% (8 of 15). The mean arterial BP of anesthetized untreated dTGR (160 ± 8 mmHg) was significantly higher than that of normotensive hRen controls (108 ± 4 mmHg), and the hearts of untreated dTGR showed pronounced left ventricular hypertrophy (Figure 1, A and B). No deaths were observed in the drug-treated groups. Compared with results for untreated dTGR, both cilazapril and losartan decreased mean arterial BP by 30 mmHg, to the level of 130 mmHg, and prevented the development of left ventricular hypertrophy (Figure 1, A and B). The combination of cilazapril and losartan completely normalized BP and myocardial size. There was a close correlation between mean arterial pressure and left ventricular hypertrophy (Figure 1C). With protocol 2, the mean arterial BP of anesthetized untreated dTGR was 157 ± 9 mmHg. Compared with results for untreated dTGR, cilazapril and losartan decreased mean arterial BP by approximately 50 mmHg (mean arterial BP of 99 ± 4 mmHg in the cilazapril-treated group and 104 ± 5 mmHg in the losartan-treated group; \( P < 0.05 \), compared with control dTGR). Cilazapril and losartan also prevented the development of left ventricular hypertrophy (3.697 ± 0.115, 2.191 ± 0.08, and 2.416 mg/g in the control, cilazapril-treated, and losartan-treated groups, respectively; \( P < 0.05 \), compared with control dTGR).

**Pressure-Natriuresis and -Diuresis Curves with Protocol 1**

Figure 2 shows pressure-natriuresis and -diuresis responses of hRen rats and dTGR. We did not include fiducial limits for...
the two untreated groups, so that the data could be more easily observed. In hRen rats, urine flow and sodium excretion averaged 13 ± 3 µl/min per g kidney wt and 1.0 ± 0.3 µmol/min per g kidney wt, respectively, at the RPP level of 100 mmHg. Increasing the RPP to 207 mmHg in these rats increased urine flow and sodium excretion to 218 ± 24 µl/min per g kidney wt and 37.5 ± 3.3 µmol/min per g kidney wt, respectively. Consequently, the slopes of the pressure-diuresis/natriuresis curves were not different, despite the rightward shift in the untreated dTGR pressure-natriuresis and -diuresis curves. Both cilazapril and losartan shifted the pressure-natriuresis and -diuresis curves back to the left by approximately 25 to 30 mmHg.

Figure 2. Relationship between renal perfusion pressure (RPP) and urine flow (A) and sodium excretion (B) for normotensive hRen rats (○), dTGR (●), and dTGR treated with either cilazapril (10 mg/kg) (■) or losartan (10 mg/kg) (▲). We did not include fiducial limits for the two untreated groups, so that the data could be more easily observed. *P < 0.05 for untreated dTGR versus dTGR treated with cilazapril or dTGR treated with losartan; values were compared at equivalent RPP levels. Pressure-diuresis and -natriuresis curves were shifted to the right for dTGR. Both cilazapril and losartan shifted the pressure-natriuresis curves leftward by approximately 25 to 30 mmHg, respectively, as RPP was increased from 69 to 124 mmHg. The pressure-natriuresis and -diuresis curves for untreated dTGR were maximally shifted rightward by 70 to 80 mmHg. In untreated dTGR, urine flow and sodium excretion averaged 13 ± 3 µl/min per g kidney wt and 1.0 ± 0.3 µmol/min per g kidney wt, respectively, at the RPP level of 100 mmHg. Increasing the RPP to 207 mmHg in these rats increased urine flow and sodium excretion to 218 ± 24 µl/min per g kidney wt and 37.5 ± 3.3 µmol/min per g kidney wt, respectively. Consequently, the slopes of the pressure-diuresis/natriuresis curves were not different, despite the rightward shift in the untreated dTGR pressure-natriuresis and -diuresis curves. Both cilazapril and losartan shifted the pressure-natriuresis and -diuresis curves back to the left by approximately 25 to 30 mmHg.

Figure 3 shows the relationships between RPP, RBF, and GFR. RBF in hRen rats averaged between 3.9 ± 0.4 and 7.5 ± 0.9 ml/min per g kidney wt. In untreated dTGR, RBF averaged between 3.3 ± 0.4 and 5.8 ± 1.0 ml/min per g kidney wt, and the curve was shifted to the right in the pressure range investigated. The GFR in hRen control rats averaged approximately 1 ml/min per g kidney wt. In untreated dTGR, the GFR was decreased, ranging between 0.56 and 0.87 ml/min per g kidney wt, and the curve was shifted to the right. Cilazapril increased RBF and GFR to 4.8 to 8.6 and to 0.85 to 1.24 ml/min per g kidney wt, respectively, so that hRen rats and cilazapril-treated dTGR were not different. Losartan had no significant effect on RBF or GFR.

Figure 4 shows fractional sodium excretion (FENa) and fractional water excretion (FEH₂O) data. In hRen rats, FENa and FEH₂O averaged 2.6 ± 1.1 and 3.6 ± 1.1%, respectively, at the RPP level of 69 mmHg and were increased to 21.1 ± 2.4 and 17.7 ± 2.8% when RPP was increased to 124 mmHg. In untreated dTGR, both curves were shifted, in parallel, approximately 15 mmHg rightward. Losartan shifted the FENa and FEH₂O curves leftward, whereas cilazapril had no significant effects on the tubular absorption of sodium and water.

The combination of cilazapril and losartan shifted the pressure-natriuresis curves leftward by approximately 70 mmHg (data not shown), which was greater than the effects observed with the cilazapril or losartan monotherapies (25 to 30 mmHg). GFR and RBF reached 1.3 ml/min per g kidney wt and 6.0 ml/min per g kidney wt, respectively, which were within the ranges found in hRen control rats. FENa and FEH₂O reached 0.6% and 0.9%, respectively, at an RPP of 70 mmHg. When RPP was increased to 102 mmHg, FENa and FEH₂O were increased to 8.0 and 5.7%, respectively. These values were not different from those observed in hRen rats with the same RPP levels. hRen rats were able to further increase FENa and FEH₂O as the RPP was increased; however, in dTGR treated with the drug combination, FENa and FEH₂O remained at the level observed at 102 mmHg as the RPP was further increased.
and sodium excretion averaged $5 \pm 0.8 \mu l/min$ per g kidney wt and $0.4 \pm 0.2 \mu mol/min$ per g kidney wt, respectively, at the RPP level of 118 mmHg. Increasing the RPP to 199 mmHg in these rats increased urine flow and sodium excretion to $130 \pm 11 \mu l/min$ per g kidney wt and $33.0 \pm 5.5 \mu mol/min$ per g kidney wt, respectively. Both cilazapril and losartan shifted the pressure-natriuresis and -diuresis curves back to the left by approximately 50 mmHg. Figure 5, C and D, shows the relationships between RPP, RBF, and GFR. In untreated dTGR, RBF averaged between $2.7 \pm 0.4$ and $4.2 \pm 1.1 \mu l/min$ per g kidney wt and GFR between 0.6 and 1.1 ml/min per g kidney wt.

Figure 3. Relationship between RPP and renal blood flow (RBF) (A) and GFR (B) for normotensive hRen rats (○), dTGR (●), and dTGR treated with either cilazapril (10 mg/kg) (■) or losartan (10 mg/kg) (▲). We did not include fiducial limits for the two untreated groups, so that the data could be more easily observed. *$P < 0.05$ for cilazapril-treated dTGR versus untreated dTGR or dTGR treated with losartan; values were compared at equivalent RPP levels. Relationships between RPP, RBF, and GFR were shifted to the right for dTGR. Cilazapril increased RBF and GFR to the levels found in normotensive rats. Losartan had no effect on RBF or GFR.

Figure 4. Relationship between RPP and fractional sodium excretion ($FE_{H_2O}$) (A) and fractional water excretion ($F_{ENa}$) (B) for normotensive hRen rats (○), dTGR (●), and dTGR treated with either cilazapril (10 mg/kg) (■) or losartan (10 mg/kg) (▲). We did not include fiducial limits for the two untreated groups, so that the data could be more easily observed. *$P < 0.05$ for losartan-treated dTGR versus untreated dTGR or dTGR treated with cilazapril; values were compared at equivalent RPP levels. $F_{ENa}$ and $FE_{H_2O}$ curves were shifted to the right for dTGR. Losartan shifted the curves leftward, whereas cilazapril did not have any effect on $F_{ENa}$ or $FE_{H_2O}$. 
Cilazapril and losartan equally increased both RBF and GFR. Figure 5, E and F, shows FENa and FEH₂O data. In untreated dTGR, FENa and FEH₂O averaged 0.3 ± 0.1 and 1.3 ± 0.5%, respectively, at the RPP level of 118 mmHg and increased to 24.0 ± 2.4 and 15.0 ± 1.8% when the RPP was increased to 199 mmHg. Losartan shifted the FENa and FEH₂O curves leftward, whereas cilazapril had no significant effects on the tubular absorption of sodium and water.

In a separate pilot study, we also examined the effects of volume depletion on pressure-natriuresis relationship in dTGR, by removing the 1% albumin solution from our infusion protocol. In volume-depleted dTGR, the pressure-natriuresis/di-
Usoresis relationship was markedly blunted. Urine flow and sodium excretion averaged 2.7 $\mu$l/min per g kidney wt and 0.2 $\mu$mol/min per g kidney wt, respectively, at the RPP level of 120 mmHg. Increasing the RPP to 147 mmHg in these rats increased urine flow and sodium excretion to 9.3 $\mu$l/min per g kidney wt and 1.0 $\mu$mol/min per g kidney wt, respectively. When the RPP was further increased to 168 mmHg, urine flow and sodium excretion were increased to 44 $\mu$l/min per g kidney wt and 9.2 $\mu$mol/min per g kidney wt, respectively. RBF averaged 2.5 to 3.7 ml/min per g kidney wt and GFR averaged 0.26 to 1.25 ml/min per g kidney wt. The hematocrit values were significantly higher (approximately 0.50) in volume-depleted dTGR, compared with non-volume-depleted TGR.

Renal Morphologic Features with Protocol 1
Histologic sections from kidneys are shown in Figure 6. The vessels of 9-wk-old untreated dTGR (Figure 6A) showed increased intimal and medial thickening, as well as hyaline deposits. The glomeruli were preserved. The tubules were

Figure 6. Histologic sections from kidneys of untreated 9-wk-old dTGR (A) and cilazapril-treated dTGR (B). In untreated dTGR, medial thickening and hyaline deposits were seen in arterioles. The interstitium showed moderate infiltration. Cilazapril treatment (10 mg/kg), as well as treatments with losartan (10 mg/kg) and the drug combination, blocked these changes. Periodic acid-Schiff reaction. Magnification, ×500.
frequently filled with proteinaceous material, and the interstitium showed moderate infiltration. Chronic treatment with cilazapril (Figure 6B), as well as with losartan and the drug combination (data not shown), was able to block the aforementioned morphologic changes in the kidney.

**RNase Protection Assays and In Situ Hybridization**

The expression of hRen, rat renin, and angiotensinogen genes in the kidney is shown in Figure 7. The results are provided in arbitrary units. Both rat and hRen mRNA levels were significantly higher in hRen rats, compared with untreated dTGR. Cilazapril and losartan treatments increased renin gene expression similarly. Rat angiotensinogen mRNA levels were decreased in untreated dTGR, compared with hRen rats. Both cilazapril and losartan increased rat and human angiotensinogen gene expression in the kidney. *In situ* hybridization experiments showed that the ACE gene was mainly localized in the tubular cells of the cortex.

![Graphs showing gene expression](image.png)

*Figure 7. Semiquantitative analysis of rat renin mRNA (A), hRen mRNA (B), rat angiotensinogen mRNA (C), and human angiotensinogen mRNA (D) expression in the kidneys of normotensive hRen rats, untreated dTGR, and dTGR treated with either cilazapril (cila) (10 mg/kg) or losartan (losa) (10 mg/kg). Both hRen and rat renin genes were downregulated in dTGR and were increased by losartan and cilazapril treatments. Drug treatments increased rat and human angiotensinogen gene expression. *P < 0.05.*
whereas the AT$_{1A}$ receptor gene was mainly expressed in the medulla (data not shown). There were no differences between the treatment groups with respect to ACE or AT$_{1A}$ receptor gene expression in the kidney (Figure 8). The expression of endothelial NO synthase mRNA in the kidney is shown in Figure 9. Cilazapril treatment significantly increased renal endothelial NO synthase gene expression, compared with losartan-treated and untreated dTGR. The endothelial NO synthase gene expression in hRen rats was lower than that in dTGR. Cilazapril and losartan did not affect inducible or neural NO synthase gene expression in the kidney (data not shown).

Discussion

The high-hRen dTGR model of hypertension features moderate hypertension with severe histologic damage in the kidneys and the heart, resulting in 50% mortality rates at 7 to 9 wk of age (1,15,16). We showed earlier that the rightward shift in the pressure-natriuresis and -diuresis curves for dTGR depends on mechanisms inherent to the kidneys themselves (2). Here we show that blockade of AngII generation with cilazapril and blockade of AT$_1$ receptors with losartan are both able to shift the pressure-natriuresis and -diuresis curves leftward in dTGR by additive mechanisms. ACE inhibition increased RBF and GFR but had no significant effects on fractional sodium or water reabsorption. On the other hand, AT$_1$ receptor blockade shifted the curves for fractional sodium and water reabsorption to the left but had relatively modest effects on RBF and GFR. Our most novel finding was that submaximal ACE inhibition and AT$_1$ receptor blockade shifted the pressure-natriuresis and -diuresis curves toward normal ranges in volume-expanded dTGR. At the doses tested (10 mg/kg), neither BP reductions nor pressure-natriuresis curve shifts were completely reversed, to normal values. In contrast, when submaximal ACE inhibition and AT$_1$ receptor blockade were combined, the BP and left ventricular mass were completely normalized, and the pressure-natriuresis curves were shifted more than with cilazapril or losartan alone. Our most novel finding was that submaximal ACE inhibition and AT$_1$ receptor blockade shifted the curves for fractional sodium and water reabsorption to the left but had relatively modest effects on RBF and GFR. Our findings indicate that both AngII-induced tubular mechanisms and AngII-mediated hemodynamic mechanisms are responsible for the resetting of pressure-natriuresis/diuresis curves in volume-expanded dTGR.
The RAS exerts multiple effects inside the kidney. AngII decreases RBF and GFR. In this respect, the sodium-retaining action of AngII is attributable in part to changes in the peritubular capillary physical forces. AngII also stimulates sodium reabsorption directly in the proximal and distal tubules (3–5). Together, these functions are responsible for the shift to higher operating arterial BP levels, as clearly demonstrated in the rat model of chronic AngII infusion (17,18) and in the mouse renin (mRen-2)27 transgenic rat model (19). We recently demonstrated that hypertension in dTGR clearly depends on the AngII-induced reduction in sodium and water excretion, which is intrinsic to the kidney (2). To clarify the importance of the RAS in pressure-dependent sodium excretion, we blocked AngII generation with cilazapril or blocked its effects on AT1 receptors with losartan. Cowley (20) showed earlier that the pressure-natriuresis mechanism is dependent on the volume state; the pressure-natriuresis relationship is markedly enhanced under conditions of volume expansion, whereas volume depletion substantially reduces the sensitivity of the pressure-natriuresis relationship. In our pilot study, we examined the effects of the volume state on the pressure-natriuresis relationship in dTGR. The volume-depleted dTGR showed marked blunting of the pressure-natriuresis relationship, compared with volume-expanded dTGR. Therefore, we emphasize that this study describes the effects of cilazapril and losartan only in volume-expanded animals, and volume expansion might have reduced the differences between treatment groups. In addition to suppressing both intrarenal and circulating AngII levels, ACE inhibitors have been reported to stimulate prostaglandin biosynthesis, diminish endothelin-1 generation, and potentiate bradykinin effects (21,22). Activation of endothelial B2 kinin receptors leads to the formation of NO, prostacyclin, and platelet-activating factor (reviewed in reference (23)). Therefore, kinins could influence renal excretory function either directly or by interactions with other endocrine and paracrine systems (24–26). We found that cilazapril, but not losartan, markedly induced endothelial NO synthase gene expression in the kidneys of dTGR, supporting the notion that kinins contribute to the cardiovascular and renal actions of ACE inhibitors. Suppression of AngII generation together with the multiple effects of cilazapril on the kallikrein-kinin system improved sodium excretion in cilazapril-treated dTGR so that, at the RPP levels investigated, sodium and water excretion were markedly increased. The fact that cilazapril shifted the pressure-natriuresis/diuresis curves without affecting FENa or FEH2O suggests that the resetting of the renal function curves by ACE inhibition may be primarily related to hemodynamic factors. This interpretation is consistent with increased RBF and GFR in cilazapril-treated dTGR and is in agreement with the view that ACE inhibitors have important effects on renal hemodynamics and GFR (27–31).

In contrast to cilazapril, chronic treatment with losartan at a submaximal dose (10 mg/kg, orally) had no significant effect on RBF or GFR in dTGR. On the other hand, the curves for FENa and FEH2O were clearly positioned to the left of the corresponding curves in cilazapril-treated dTGR. Our findings indicate that a net decrease in tubular sodium reabsorption might have been responsible for the improvements in sodium excretion and BP reduction in losartan-treated dTGR. Consistent with our findings, other authors also suggested that the antihypertensive effects of long-term AT1 receptor blockade are related to direct inhibitory effects on sodium reabsorption in renal tubular cells (5,6). However, when losartan was administered to dTGR at a significantly higher dose (30 mg/kg, orally), not only FENa and FEH2O, but also RBF and GFR were markedly increased, indicating that the effects of AT1 receptor blockade on renal hemodynamics are clearly dose-dependent and may occur only at very high doses. Consistent with our findings, Wang et al. (18) recently showed that in rats with AngII-induced hypertension, chronic treatment with high doses of losartan prevented the AngII-induced decreases in GFR and RBF and the AngII-induced increases in BP. However, we emphasize that although both cilazapril and losartan markedly improved renal hemodynamics when administered at higher doses, impaired autoregulation persisted. The reasons for this relatively poor autoregulation in dTGR in this study and our earlier report (2) remain unclear.

We confirmed our previous findings (1,2) that both transfected genes are expressed in the kidney. Both hRen and rat renin genes were physiologically regulated in 9-wk-old hypertensive dTGR; this is similar to the results we reported for 6-wk-old dTGR (2). The lower expression of hRen and rat renin genes in dTGR is probably related to higher BP and higher AngII levels in these rats. The expression of both renin genes was also increased after long-term treatment with cilazapril and losartan, in parallel with the drug-induced BP reduction in these rats. These observations are different from those reported for the (mRen-2)27 transgenic rat model. In those transgenic rats, which bear the salivary (mRen-2)27 gene, the mRen gene is expressed in the kidney but is relatively impermeable to even lifelong ACE inhibition, with normalization of BP (14).

Rat angiotensinogen gene expression in the kidney was lower in untreated dTGR, compared with control animals. Moreover, both cilazapril and losartan increased rat as well as human angiotensinogen gene expression in the kidney. Our findings are consistent with results from previous studies (14,32,33) and suggest that AngII and BP are physiologic regulators of angiotensinogen gene expression in the kidney. This study is the first to describe rat ACE and AT1A receptor gene expression in dTGR. We found no differences between normotensive control rats and dTGR in the expression or localization of these genes in the kidney. Neither chronic ACE inhibition nor AT1 receptor blockade had any effect on the expression of these genes. We did not measure renal AT1 receptor protein density in this study. Therefore, we cannot speculate on whether the high circulating AngII levels in dTGR are able to reduce the density of AT1 receptors in the kidneys without altering the renal expression of the AT1A receptor gene, as shown by Sechi et al. (34).

In summary, our experiments indicate that pressure-natriuresis/diuresis curves are shifted to higher RPP ranges in dTGR, compared with normotensive control rats, and that inhibition of AngII generation by cilazapril or blockade of AT1 receptors by
losartan is accompanied by a shift of these renal function curves to lower operating arterial BP levels. When administered at submaximal doses, the two drugs exert similar effects on systemic BP and pressure-natriuresis/diuresis curves; however, cilazapril improved sodium and water excretion mainly by increasing RBF and GFR, whereas losartan decreased tubular sodium and water reabsorption without markedly affecting renal hemodynamics. A combination of ACE inhibition and AT1 receptor blockade induced an additive effect. Our results suggest that the hypertensive rightward shift in the pressure-natriuresis curve in dTGR is dependent on AngII-induced changes in renal hemodynamics, as well as on disturbances in tubular function.

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