Pressure-Natriuresis and -Diuresis in Transgenic Rats Harboring both Human Renin and Human Angiotensinogen Genes

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Abstract. The hypertensive double transgenic rat harboring both the human renin and human angiotensinogen genes (dTGR) offers a unique opportunity to study the human renin-angiotensin system in an experimental animal model. Since nothing is known about the control of sodium and water excretion in these rats, this study was performed to compare pressure-natriuresis relationships in hypertensive dTGR and normotensive control rats harboring only the human renin gene (hREN), in order to determine how the pressure-natriuresis relationship is reset in hypertensive dTGR. To differentiate between extrinsic and intrinsic renal mechanisms, experiments were performed with and without renal denervation, and with and without infusions of vasopressin, norepinephrine, 17-OH-corticosterone, and aldosterone. Human and rat angiotensinogen and renin mRNA expression were also determined. In hREN without controlled renal function, urine flow and sodium excretion increased from 13 to 169 μA/mm per g kidney wet weight (kwt) and from 1 to 30 μmol/min per g kwt, respectively, as renal perfusion pressure was increased from 67 to 135 mmHg. Renal blood flow (RBF) and GFR ranged between 3 to 7 and 0.9 to 1.5 ml/min per g kwt. In dTGR, pressure-natriuresis-diuresis relationships were shifted approximately 40 mmHg rightward. RBF was lower in dTGR than in hREN; GFR was not different. In dTGR with neurohormonal factors controlled, RBF was decreased and pressure-natriuresis-diuresis curves were not different compared to dTGR curves without these interventions. By light microscopy, the kidneys of these 6-wk-old dTGR and hREN rats were normal and indistinguishable. Both human and rat angiotensinogen mRNA were expressed in the kidneys of dTGR. The two renin mRNA were decreased in dTGR, indicating a physiologic downregulation of renin gene expression by high BP. It is concluded that the renal pressure-natriuresis mechanism is reset toward higher pressure levels in dTGR and participates in the maintenance of hypertension. The reduced excretory function in dTGR depends on hREN and human angiotensinogen gene expression and is intrinsic to the kidney as opposed to extrarenal regulators.
sodium and water excretion, we performed our studies under conditions in which neuronal and hormonal influences were controlled by renal denervation and infusions of aldosterone, 17-OH-corticosterone, norepinephrine, as well as under conditions without these maneuvers. We deliberately studied the rats at an early age to avoid the confounding influence of renal structural changes.

Materials and Methods

Experiments were conducted in seventeen 6-wk-old male dTGR harboring hREN and hAogen, and weighing 181 ± 26 g. As controls, 17 male normotensive rats harboring only hREN and weighing 185 ± 41 g were used. These rats are termed hREN rats. The human renin construct used to generate transgenic animals comprised the entire genomic human renin gene (10 exons and 9 introns) with 3.0 kb of the 5′ promoter region and 1.2 kb of 3′ additional sequences. hAogen is a genomic construct of the entire human angiotensinogen gene (5 exons and 4 introns) with 1.3 kb of 5′ flanking and 2.4 kb of 3′ flanking sequences. In preliminary studies, we found no differences in renal function or BP in hREN rats or in rats harboring only the hAogen gene compared with normal Sprague Dawley rats. Therefore, we used only one normotensive strain (hREN) as controls. The animals were obtained from Biological Research Laboratories (Füllinsdorf, Switzerland). The rats were allowed free access to standard 0.3% sodium and water ad libitum. The experimental protocol was approved by the local Council on Animal Care, whose standards correspond to those of the American Physiological Society.

The rats were prepared as described previously (8). Briefly, they were anesthetized with an intramuscular injection of 35 mg/kg ketamine (Parke-Davis, Berlin, Germany) and an intraperitoneal injection of 65 mg/kg inactin (Res Biochemical, Natick, MA) and placed on a heated table to maintain body temperature 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 also around the aorta below the left renal artery for later occlusion. In this manner, RPP could be varied above and below control values so that for a representative range relationships between the urinary excretion of sodium and water and renal perfusion pressure could be determined. During preparation, the rats were infused with a 0.9% saline solution containing 1% bovine serum albumin at a rate of 50 μl/min per 100 g body wt. Thereafter, inulin (20 mg/ml; Sigma Chemical Company, St. Louis, MO) and para-aminohippurate (PAH) (1.2 mg/ml; Merck Sharp and Dohme, West Point, PA) were included in the infusion for measurement of GFR and renal plasma flow (RPF), and the infusion rate was reduced to 33 μl/min per 100 g body wt.

Animal Protocols

Protocol 1: Pressure-Natriuresis and -Diuresis without Neuronal and Hormonal Control. Experiments were performed in eight hREN rats weighing 195 ± 27 g and six dTGR rats weighing 174 ± 37 g. The rats were prepared as described above but the left kidney was not denervated and the hormone cocktail was not added to the infusion. After surgical preparation and an equilibration period of 45 to 60 min, RPP was lowered to approximately 67 mmHg in hREN rats and 108 mmHg in dTGR rats. After an additional 30-min equilibration period, urine flow, sodium excretion, GFR, and renal blood flow were determined in two 20- to 30-min collecting periods, depending on the magnitude of the urine flow. The supra-aortic occluder was then released to increase RPP to approximately 102 mmHg in hREN rats and 142 mmHg in dTGR rats. Again, urine and plasma samples were taken after a 25- to 30-min equilibration period. RPP was finally increased to approximately 135 mmHg in hREN rats and 196 mmHg in dTGR rats. After a 10- to 15-min equilibration period, urine and plasma samples were collected in two 5-min periods depending on the high urine flow at this RPP level.

To demonstrate the importance of AngII for the shift in pressure-diuresis-natriuresis relationships in dTGR rats (n = 3), we administered the AT1 antagonist CV11794 (0.1 mg/kg intravenously, 100 μl/100 g body wt) (Takeda Chemical Industries) as a bolus. After surgical preparation and after the equilibration period pressure-diuresis-natriuresis relationships were determined as described above, we compared the values to the pressure-diuresis-natriuresis relationships of an additional group of untreated dTGR rats (n = 4). Because we had the necessary statistical power, we did not need to study larger numbers of animals.

Protocol 2: Pressure-Natriuresis and -Diuresis with Neuronal and Hormonal Control. Experiments were performed in nine hREN rats weighing 176 ± 52 g and 11 dTGR rats weighing 185 ± 20 g. In these rats, the left kidney was denervated by stripping the visible nerves and swabbing the renal artery and vein with 10% phenol in ethanol. In these experiments, the circulating levels of water- and sodium-retaining hormones were also fixed by an infusion of norepinephrine (333 ng/kg per min), aldosterone (66 ng/kg per min), 17-OH-corticosterone (33 μg/kg per min), and vasopressin (0.17 ng/kg per min) (all from Sigma). The combined procedures of denervation and increased hormone levels are subsequently referred to as neurohormonal clamping. After surgery and equilibration, the RPP was lowered to approximately 80 mmHg in hREN rats and 113 mmHg in dTGR rats. After a 30-min equilibration period, urine and plasma samples were collected. By releasing the supra-aortic occluder, RPP was increased by approximately 110 mmHg in hREN rats and 147 mmHg in dTGR rats. Again, samples were taken after a 25- to 30-min equilibration period. Finally, RPP was increased to approximately 143 mmHg in hREN rats and 190 mmHg in dTGR rats by tying off both mesenteric and celiac arteries, as well as the abdominal aorta. After an equilibration of 10 min, samples were collected in two 5-min periods.

BP Measurement, Inulin, PAH Clearance, and Plasma Aldosterone Levels

Mean arterial pressure was continuously measured throughout the experiment and recorded on a computer system (Technical Services Equipment, Bad Homburg, Germany). Representative mean arterial pressure values were calculated before starting pressure-natriuresis experiments to characterize BP levels of hREN rats and dTGR and for each urine collection period by averaging all recorded values during that period. Urine flow was determined gravimetrically. Inulin and PAH concentrations of urine and plasma samples were determined according to methods outlined elsewhere (9,10). GFR was calculated as the urine-to-plasma inulin concentration ratio multiplied by urine flow. RBF was calculated as RPF/(1-Hct). 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 kidney wet weight. Plasma aldosterone levels were determined by RIA (Diagnostic Products Corp., Los Angeles, CA) in additional groups of 6-wk-old hREN (n = 11) and dTGR (n = 14) rats in which blood was drawn from the ocular plexus.
Morphology and in Situ Hybridization

For conventional morphology, 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 (2). The tissue was examined without knowledge of the rat's identity.

RNase Protection Assay. For the RNase protection assay, the kidneys were deep frozen. Total RNA was isolated from the kidneys, and the RNase protection assay was performed with an Ambion RPA III kit (ITC Biotechnology, Austin, TX), according to the manufacturer's description as described previously (4). For the detection of rat and human renin and rat and human angiotensinogen mRNA, the same probes were used as described by Bohlender et al. (4).

In Situ Hybridization. To prepare digoxigenin-11-UTP (DIG-11-UTP) (Boehringer, Mannheim, Germany)-labeled RNA probes (11), 1 μg of linearized plasmid DNA was incubated in a transcription buffer (40 mM Tris-HCl, pH 8.0, 6 mM MgCl₂, 10 mM NaCl, 10 mM dithiothreitol, and 2 mM spermidine) with 750 μmol ATP, CTP, and GTP; 150 μmol UTP; 600 μmol DIG-11-UTP; 1 μg/μl RNase inhibitor (Boehringer); and 2 μg/μl T7 or SP6 RNA polymerase (Boehringer) at 37°C for 120 min. The DNA template was digested by addition of 20 ng/μl RNase-free DNase I (Boehringer) for 20 min at 37°C in the presence of 1 μg/μl RNase inhibitor. The incubation mixture was ethanol-precipitated, and the RNA concentration was measured in an ultraviolet spectrophotometer at 260 nm. The labeling quality was checked by separating the probes via a denaturating formaldehyde gel electrophoresis, transferring the RNA onto a Nylon membrane (Nytran N, Schleicher & Schull, Dassel, Germany), and detection of the digoxigenin by an alkaline phosphatase-conjugated antibody (Boehringer). For in situ hybridization, the kidneys were snap-frozen in isopentane (−35°C), sectioned at a 10-μm thickness in a cryostat (Leica Frigocat, Wetzlar, Germany), and mounted onto silane-coated slides. The hybridization procedure is described elsewhere (11). In brief, the sections were fixed for 5 min in 3% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.0, followed by a rinse in PBS and two washes for 5 min in sterile water. The slides then underwent a deproteination in 0.2 M HCl for 20 min. After two short washing steps in PBS, the slides were acetylated for 20 min in 0.1 M triethanolamine (pH 8.0)/0.25% acetic anhydride. The slides were washed twice for 3 min in PBS, dehydrated in graded ethanol, and air dried. Subsequently, 150 μl of prehybridization buffer (50% denized formamide, 50 mM Tris-HCl, pH 7.6, 25 mM ethylenediaminetetra-acetic acid [EDTA], pH 8.0, 20 mM NaCl, 0.25 mg/ml yeast tRNA, and 2.5× Denhardt's solution [0.05% Ficoll, 0.05% polyvinylpyrrolidone, 0.05% bovine serum albumin]) was applied to each section, and the slides were incubated in a humidified chamber at 37°C for 2 to 4 h.

After draining the prehybridization buffer off the slides, hybridization buffer (50% denized formamide, 20 mM Tris-HCl, pH 7.6, 1 mM EDTA, pH 8.0, 0.3 M NaCl, 0.2 M dithiothreitol, 0.5 mg/ml yeast tRNA, 0.1 mg/ml poly(A) RNA, 1× Denhardt's solution, and 10% dextran sulfate) containing either labeled antisense RNA or sense RNA (each 2.5 ng/μl) for control experiments was put onto slides. The sections were covered with siliconized coverslips and incubated at 37°C for 16 to 18 h in a humidified chamber. Coverslips were removed by immersing the slides for 30 min in 1× SSC (0.3 M NaCl, 0.03 M Na-citrate) at 48°C, followed by washing in 0.5× SSC/50% formamide at 48°C for 4 h (the washing solution was changed every 60 min), and then rinsed in 1× SSC followed by two times for 5 min each in buffer I (100 mM Tris-HCl, 150 mM NaCl, pH 7.5). The slides were then incubated for 30 min at room temperature with 5% normal sheep serum, 0.3% Triton X-100 in buffer I. After a short rinse in buffer I, 200 μl per section of sheep anti-DIG-alkaline phosphatase conjugate: (1:500 in buffer I, 1% normal sheep serum, 0.3% Triton X-100; Boehringer) was applied and incubated at room temperature for 3 h in a humidified chamber. The slides were then washed 3 × 10 min in buffer I, rinsed in buffer III (100 mM Tris-HCl, 100 mM NaCl, 50 mM MgCl₂, pH 9.5), and equilibrated with buffer III for 10 min. The slides were then rinsed in distilled water and air dried. Subsequently, they were counterstained with neutral red (nuclear stain) and covered with glycerine-gelatin (Merck).

Statistical Analyses

Data are presented as mean ± SEM. Statistically significant differences in mean values were tested by two-way ANOVA for repeated measures and the Duncan multiple range test. A value of P < 0.05 was considered statistically significant.

Results

Protocol 1: Pressure-Natriuresis and -Diuresis without Neuronal and Hormonal Control

Mean arterial pressure before the pressure-natriuresis experiments averaged 104 ± 6 mmHg in hREN rats and 139 ± 25 mmHg in dTGR rats. The animals were young and the hypertension was in the process of development in dTGR rats. Plasma aldosterone levels in hREN rats were 0.5234 ± 0.072 nmol/L, whereas in dTGR rats these values were 1.2604 ± 0.139 nmol/L. Thus, dTGR showed a 2.4-fold increase of aldosterone concentrations compared with control rats. Figure 1A shows pressure-diuresis and -natriuresis responses of hREN rats and dTGR rats without clamping of neuronal and hormonal influences on kidney function. In hREN rats, urine flow and sodium excretion increased from 13.3 ± 3.1 to 169.2 ± 29.3 μl/min per g kwt and from 1.2 ± 0.4 to 29.7 ± 4.6 μmol/min per g kwt, respectively, as RPP was increased from 67 to 135 mmHg. The pressure-diuresis-natriuresis curves of dTGR were shifted rightward by approximately 40 mmHg. In these rats, urine flow and sodium excretion averaged 8.9 ± 1.7 μl/min per g kwt and 0.8 ± 0.2 μmol/min per g kwt, respectively, at the RPP level of 108 mmHg. Increasing the RPP to 196 mmHg in these rats was also accompanied by a large increase of urine flow and sodium excretion to 208.8 ± 18.6 μl/min per g kwt and 36.8 ± 3.1 μmol/min per g kwt, respectively. Consequently, the slopes of the pressure-diuresis-natriuresis curves were not different, independent of the rightward shift in dTGR pressure-diuresis-natriuresis curves.

Figure 2A shows the relationships between RPP and RBF and GFR. RBF in hREN rats averaged between 2.7 ± 0.2 and
Pressure-Natriuresis in Double Transgenic Rats

Figure 1. Relationship between renal perfusion pressure (RPP) and urine flow and sodium excretion in normotensive human renin (hREN) rats (open symbols) and hypertensive, double transgenic (rats harboring the hREN and human angiotensinogen [hAogen] genes: double transgenic rats [dTGR]) rats (closed symbols) without (A) and with (B) clamping of neurohormonal systems. *P < 0.05, values compared to equivalent RPP levels. Pressure-diuresis and -natriuresis curves were shifted toward the right in dTGR, regardless of whether neurohormonal systems influencing sodium and water excretion were controlled.

Figure 3A shows fractional sodium and water excretion. Fractional sodium and water excretion averaged 0.8 ± 0.2 and 1.6 ± 0.4% at the RPP level of 67 mmHg in hREN rats and increased to 14.7 ± 2.2 and 12.1 ± 2.1% when RPP was increased to 135 mmHg. In dTGR rats, both curves were shifted in parallel approximately 40 mmHg rightward, as it was already observed for urine flow and sodium excretion.

AT1 receptor blockade improved sodium and water excretion in dTGR rats. The pressure-natriuresis and pressure-diuresis curves were regularly shifted leftward by 20 mmHg, compared with controls (data not shown). The results were reproducible so that the statistical analysis showed a significant result already when three dTGR rats and four control rats were compared.

Protocol 2: Pressure-Natriuresis and -Diuresis with Neuronal and Hormonal Control

Mean arterial pressure averaged 105 ± 27 mmHg in hREN rats, and 141 ± 33 mmHg in dTGR rats. These values were not different from BP measured before the pressure-natriuresis experiments in protocol 1.

Figure 1B shows the pressure-diuresis and -natriuresis responses of hREN rats and dTGR rats with controlled neuronal and hormonal factors. The pressure-diuresis-natriuresis rela-
Figure 2. Relationship between RPP and renal blood flow (RBF) and GFR in hREN rats (open symbols) and dTGR (closed symbols) without (A) and with (B) clamping of neurohormonal systems. * P < 0.05, values compared to equivalent RPP levels. Relationships between RPP and RBF were shifted toward the right in dTGR. GFR was not different between both groups.

Figure 3B shows fractional sodium and water excretion. Fractional sodium and water excretion averaged 2.0 ± 0.5 and 1.3 ± 0.3% at the RPP level of 80 mmHg in hREN rats and rose to 17.6 ± 1.9 and 12.8 ± 2.5% when RPP was increased to 143 mmHg. In dTGR rats, both curves were shifted parallel to higher levels of RPP.

Hematocrit values of all groups ranged between 41.5 and 44.8%, indicating a good state of hydration. Plasma sodium concentrations were similar in hREN and dTGR rats and av-
Figure 3. Relationship between RPP and fractional water and sodium excretion in hREN rats (open symbols) and dTGR (closed symbols) without (A) and with (B) clamping of neurohormonal systems. * \( P < 0.05 \), values compared to equivalent RPP levels. Fractional sodium and water excretion curves were shifted toward the right in dTGR, regardless of whether the neurohormonal systems influencing sodium and water excretion were controlled.

eraged 142 mmol/L. Plasma potassium was slightly decreased in dTGR compared with hREN rats and averaged 4.3 and 3.8 mmol/L, respectively. By light microscopy, the kidneys of hREN and dTGR rats were normal and morphologically indistinguishable.

**RNase Protection Assay**

To verify the human and rat renin and angiotensinogen gene expressions, we performed RNase protection assays as shown in Figure 4. The quantification is given in arbitrary units (AU). Rat renin mRNA (top panel, left) was significantly \( (P < 0.05) \) higher in hREN rats (0.1039 ± 0.0123 AU) than in dTGR rats (0.0111 ± 0.0028 AU). The human renin gene expression was also higher in hREN rats (0.083 ± 0.037 AU) than in dTGR rats (0.024 ± 0.0065 AU), but more variable, so that with the number of assays performed the significance is marginal \( (0.05 < P < 0.1) \). Rat angiotensinogen mRNA was not different between the two strains and leveled to 0.09 AU. Human angiotensinogen mRNA was only found in dTGR.

**In Situ Hybridization**

Figure 5 demonstrates the localization of the human and rat renin messages in the kidneys of hREN rats and dTGR. The renin mRNA was located in the afferent arteriole in both strains, but was much more prominent in the arterioles of hREN rats (top panel, left) compared with dTGR rats (top panel, right), where the message was harder to find. The human renin mRNA was also expressed in the afferent arteriole, but to a lesser extent than the rat renin message. The rat renin message was even less abundant in the dTGR rat (bottom panel,
Discussion

As in every genetic model of hypertension studied to date, our results indicate that the relationship between arterial pressure and renal sodium and water excretion is shifted toward higher renal perfusion pressure ranges in dTGR rats, compared with normotensive hREN controls. Controlling non-RAS neuronal and hormonal influences had no effect on sodium and water excretion in dTGR. The plasma aldosterone levels of dTGR were 2.4-fold higher than hREN rats; however, controlling for this difference with exogenous aldosterone administration sufficient to raise circulating aldosterone values sixfold higher than observed in rats receiving usual chow did not influence the difference in pressure-natriuresis and diuresis observed between the two strains (7). Thus, the locally acting human RAS is implicated directly, since possible indirect effects via the sympathentic nervous system, aldosterone, 17-OH-corticosterone, or vasopressin-related actions were controlled. Because dTGR showed no signs of nephrosclerosis,
Figure 5. The localization of human and rat renin mRNA in the kidneys of dTGR and hREN rats is demonstrated by nonradioactive in situ hybridization. Rat renin mRNA (arrow) was expressed in the afferent arterioles of hREN rats. This mRNA was very abundant and it spread into the afferent arteriole (top panel, left). In contrast, the rat renin mRNA (arrow) was repressed in the juxtaglomerular apparatus in kidneys of dTGR (top panel, right). The bottom panel demonstrates the human renin transgene expression (arrow) in hREN rats (left) and dTGR (right) in the appropriate place in the afferent arteriole. The sections are counterstained with neutral red. g, glomerulus. Magnification, ×500.

only functional changes of the human RAS caused the rightward shift of pressure-diuresis-natriuresis relationships in dTGR rats.

High levels of circulating human renin and human angiotensinogen in dTGR rats were described by our group in an earlier communication (4). We demonstrated the expression of human angiotensinogen and human renin in tubules and in the afferent arteriole of TGR, respectively. Both human and rat renin genes are physiologically regulated, as is seen by the lower expression in dTGR probably related to their higher BP values. Because transfection of the hREN gene alone into Sprague Dawley rats did not affect BP or pressure-diuresis-natriuresis relationships, the BP increase and the rightward shift in renal function curves are clearly related to both the hREN and hAogen genes. These data underscore the species-specificity of the renin-angiotensinogen interaction. Clearly, human renin has no effect on rat angiotensinogen. In this regard, the hREN gene-related effect is different compared with the effects of the mouse Ren-2 gene in Sprague Dawley rats. The (mRen-2)27 TGR rats develop severe hypertension because mouse renin cleaves rat angiotensinogen with even greater proclivity than rat renin (1–3). The mouse renin transgene in these rats is expressed in the kidney and was found to be responsible for the rightward shift in pressure-diuresis-natriuresis relationships (3). The rightward shift in pressure-natriuresis is pivotal for hypertension development in these rats (2,12).

In (mRen-2)27 TGR rats, changes in fractional sodium and water excretion paralleled the pressure-diuresis-natriuresis relationships, suggesting that the reduced ability of these rats to excrete sodium and water may have been primarily related to increased tubular reabsorption (2,3,12). In the current study, FE\textsubscript{Na} and FE\textsubscript{H\textsubscript{2}O} curves were also shifted toward the right in dTGR. Both in (mRen-2)27 TGR rats studied earlier (2) and also in the dTGR rats currently studied, we were able to shift pressure-diuresis-natriuresis curves leftward toward normal by administering an AT\textsubscript{1} receptor blocker. Therefore, we assume that derangements in sodium and water excretion in dTGR are also largely AngII-dependent and are due to elevations in tubular reabsorption of sodium and water for any given level of renal perfusion pressure. AngII is able to influence tubular reabsorption of sodium and water directly (13–15).
In addition to tubular reabsorption, AngII could also influence pressure-natriuresis. Recent evidence suggests that the renal tubule is able to concentrate AngII to higher values than those observed in the circulating blood. This AngII cannot be attributed to filtration, and thus must be a function of tubular cell secretion and perhaps local production. The human Aogen and rat Aogen mRNA we detected in the tubules of the renal cortex is shown in the bottom panel in the same rat. The same distribution of both messages was present in the tubules of the cortical layer; however, the human angiotensinogen gene expression was more abundant and spread to more tubules than the rat angiotensinogen mRNA. The sections are counterstained with neutral red. g, glomerulus. Magnification, ×500.

Recent evidence suggests that the renal tubule is able to concentrate AngII to higher values than those observed in the circulating blood. This AngII cannot be attributed to filtration, and thus must be a function of tubular cell secretion and perhaps local production. The human Aogen and rat Aogen mRNA we detected in the tubules of dTGR could be the substrate for local AngII generation in the tubular cells. High AngII tubular values have been described in 2K 1C Goldblatt rats, AngII infused rats, and recently in transgenic rats harboring the mRen-2 transgene. Although we did not measure AngII values directly, we speculate that they were elevated and could have induced the rightward shift in pressure-natriuresis. This interpretation could explain why intrinsic rather than extrinsic mechanisms are particularly important in this model. Furthermore, both acute and chronic experiments in rats have shown that AngII influences pressure-natriuresis. In addition to tubular reabsorption, AngII could also have influenced tubuloglomerular feedback (23,24) or renal hemodynamics (20,25). Renal blood flow curves were shifted toward the right in dTGR compared with controls. This finding may also indicate that AngII influenced sympathetic vascular tone in dTGR (26,27).

AngII has distinct effects on renal cortical and papillary blood flow (28). In (mRen-2)27 TGR rats, bolus volume expansion resulted in decreased medullary blood flow (29). Medullary blood flow is believed to play an important role in sodium excretion and the related development and maintenance of hypertension (30). AngII-dependent changes in medullary blood flow may also have contributed to the rightward shift in pressure-diuresis-natriuresis relationships in dTGR. Surprisingly, we did not find large differences in GFR between hREN rats and dTGR. This observation indicates that dTGR rats were able, independent of the overly active renin-angiotensin system and hypertension, to maintain GFR by changes in renal vascular resistance. The absence of glomerular damage at this early stage of the hypertension may be another factor that supported the well preserved autoregulation of GFR in dTGR rats.

In contrast to deoxycorticosterone acetate-salt hypertensive Sabra rats, in which we found that pressure-natriuresis was more affected by extrinsic than intrinsic renal factors, we found no differences in pressure-dependent sodium and water excretion in dTGR rats, regardless of whether neural and hormonal factors to the kidney were controlled. This finding underscores the overwhelming role of the hREN and hAogen genes and the pivotal role of the kidney for hypertension production in dTGR rats. With renal denervation, we ruled out the possibility that changes in renal sympathetic tone are an important factor for sodium and water excretion in dTGR rats. Such efforts are highly important in other forms of hypertension, independent of renal vascular tone. Furthermore, we were able to exclude an important role for vasopressin, aldosterone, circulating norepinephrine, and 17-OH-corticosterone in dTGR pressure-natriuresis relationships.

Recent work on mice harboring both the human renin and human angiotensinogen gene has been reported, indicating that these mice have a 40 mmHg higher level of BP than control mice and develop striking nephrosclerosis at 18 mo of age (32). Murine renin was located exclusively in the juxtaglomerular apparatus, whereas human renin was more widespread in these animals. Because pressure-natriuresis and -diuresis measurements can now be practically performed in mice, the opportunities for comparison are possible. The relevance of transgenic rodent models to the study of hypertension has been studied by Merrill et al. (34). They emphasize that transgenic models are helpful in assessing the biochemical and physiologic importance of candidate genes. Admittedly, transgenic models such as those we used are contrived and appear distant from the human condition. Nevertheless, the model will allow the study of local actions, such as an intrarenally induced AngII-related shift in pressure-natriuresis as we demonstrated here.

We conclude that pressure-diuresis and pressure-natriuresis relationships are shifted toward higher levels of renal perfusion.
pressure in hypertensive dTGR rats compared with hREN controls. As the parallel shifts in fractional sodium and water excretion indicate, tubular reabsorption of sodium and water was increased in dTGR rats. The reduced sodium and water excretory capacity of dTGR is intrinsic to the kidney, and probably depends on an overactive intrarenal human RAS. Although we cannot rule out the direct, structurally induced effect of increased BP on pressure-diuresis-natriuresis curves, we believe that derangements in AngII-dependent sodium and water excretion are primarily involved in the development of human renin-induced hypertension. Our results with AT1 receptor blockade support that view. Finally, double transgenic models permit pharmacologic investigations on human systems in experimental animals. Rat and mice models are likely to be complementary in that regard.

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References