High Human Renin Hypertension in Transgenic Rats

Jurgen Bohlender, Akiyoshi Fukamizu, Andrea Lippoldt, Tatsuo Nomura, Rainer Dietz, Joel Ménard, Kazuo Murakami, Friedrich C. Luft, Detlev Ganten

Abstract We developed a model of spontaneously high human renin hypertension in the rat by producing two transgenic strains, one for human angiotensinogen with the endogenous promoter and one for human renin with the endogenous promoter. Neither transgenic strain was hypertensive. These strains were then crossed, producing a double transgenic strain. The double transgenic rats, both males and females, developed severe hypertension (mean systolic pressure, 200 mm Hg) and died after a mean of 55 days. The rats had a human plasma renin concentration of 269 ± 38 ng Ang I/mL per hour, plasma renin activity of 177 ± 170 ng Ang I/mL per hour, rat angiotensinogen concentration of 1.49 ± 1 µg Ang I/mL, and human angiotensinogen concentration of 78 ± 39 µg Ang I/mL (n = 49). Control rats had plasma renin activity of 3.7 ± 3.9 ng Ang I/mL per hour and rat angiotensinogen of 1.32 ± 0.16 µg Ang I/mL. Angiotensinogen transgene expression by RNase protection assay was ubiquitously present but most prominent in liver. Renin transgene expression was high in kidney but absent in liver. The rats featured severe cardiac hypertrophy, with increased cross section of cardiomyocytes but little myocardial fibrosis. The kidneys showed atrophic tubules, thickened vessel walls, and increased interstitium. Both the angiotensin-converting enzyme inhibitor lisinopril and the specific human renin inhibitor remikiren lowered blood pressure to normal values. Double transgenic mice have been developed that exhibit features quite similar to those described here, their gene expressions are similar. The specificity of rodent and human renin is similarly documented. Although many elegant physiological studies can now be done in rats, rats nevertheless offer flexibility, particularly in terms of detailed cardiac and renal physiology and pharmacology. We conclude that this double transgenic strain will facilitate simultaneous investigation of genetic and pathophysiological aspects of renin-induced hypertension. The fact that human renin can be studied in the rat is a unique feature of this model (Hypertension. 1997;29[part 2]:428-434.)

Key Words • hypertension, experimental • rats, transgenic • renin • angiotensin • hypertrophy • nephrosclerosis

Human renin (hREN) cleaves rAOGEN slowly and poorly. Similarly, hAOGEN is converted to Ang I by hRFN but not by rRFN. To study hREN in an animal model, we developed a transgenic rat [{TGR(hAOGEN)1623}] that harbors the hAOGEN gene. When infused with hREN, this otherwise normotensive rat becomes severely hypertensive. Under the condition of chronic infusion with hREN, the rat is suitable for study of hREN inhibitors, which otherwise have no effect in the rat. However, for study of the human renin-angiotensin system in the rat for periods ranging from weeks to months, the minipump infusion model has major shortcomings, making it not suitable for studying mechanisms of cardiac hypertrophy, hypertensive nephrosclerosis, or central nervous system damage. To circumvent this problem, we have crossed the TGR(hAOGEN) with a TGR harboring the hREN gene. This TGR(hAOGEN)10J was developed in Japan. Information on this rat has not yet been published. Offspring from this cross harbor both transgenes and therefore have all the necessary components of the human renin-angiotensin system for generation of Ang II. We measured components of the renin-angiotensin system in these rats, we studied the expression of both transgenes, we observed blood pressure and end-organ damage; we determined survival, and we tested an orally effective hREN inhibitor. We conclude that this TGR(hAOGEN-hREN) is a novel, double transgenic model of high hREN malignant hypertension. The animal is highly suitable for physiological and pharmacological experimentation.

Methods

Animals

Male Sprague-Dawley rats homozygous for the complete human genomic angiotensinogen gene [TGR(hAOGEN)1623] and nontransgenic Sprague-Dawley rats weighing 230 to 350 g were used for the experiments. The transgenic line and its characteristics have been described elsewhere. Briefly, TGR(hAOGEN) show high hAOGEN gene expression in the liver, kidney, heart, aorta, brain, and adrenal glands. Their plasma hAOGEN concentrations exceed endogenous AOGEN concentrations by 50- to 100-fold. A new TGR carrying the entire hREN gene DNA with 10 exons and 9 introns spanning 15 kb including 3 kb of its 5' flanking promoter region and a 1.2-kb 3' flanking region was created by oocyte microinjection methods on outbred Sprague-Dawley background, as published elsewhere. A new homozygous line, TGR(hREN)10J, was established from a male founder rat with appropriate backcrosses. The TGR were normotensive All...
rats were kept under standard conditions at 24±2°C, fed a commercial rat chow (No. C-1000, Altromin) containing 0.2% sodium by weight, and had free access to tap water.

Six male homozygous TGR(hAOGEN) were mated with two female homozygous TGR(hREN) each. Thirty-four male and 15 female offspring heterozygous for both transgenes were obtained. The presence of the two transgenes was checked by Southern blotting. Drinking water was measured daily and body weight determined twice a week. When signs of illness were observed, rats were killed by an overdose of intraperitoneal ketamine with xylazine, and organs were removed. Blood pressure was measured by the tail-cuff method with rats under light ether anesthesia. The wet weight (grams) of the hearts was determined. Nine male and 3 female age-matched Sprague-Dawley rats were used for anesthetized Sprague-Dawley rats were used as controls and killed in parallel with the double TGR. Six female double TGR received the angiotensin-converting enzyme inhibitor lisinopril (10 mg/L) for 7 days in their drinking water. Six female double TGR received the hREN inhibitor remikren (100 mg/L) in drinking water (Ro 42-5892, Hoffmann-La Roche). The remikren concentration was chosen on the basis of the bioavailability of the compound.

Blood samples (0.6 mL) were drawn by jugular venous puncture before and at the end of experiments or at the time of death. For this purpose, the rats were anesthetized with ketamine-xylazine (15 and 5 mg/kg body wt IP). Anticoagulation was with Na2EDTA (6.25×10−5 mol/L of blood) hAOGEN and hREN concentrations, total PRA, human- and rat-specific PRCs (hPRC and rPRC), and human plasma renin concentrations were determined by enzyme-kinetic assay. For a detailed analysis of gene expression, 13 organs from one TGR(hREN) and one double TGR were removed, snap-frozen in liquid nitrogen, and stored at −70°C. Tissue was also processed for formalin for histology.

PRA, hPRC, rPRC, hAOGEN, and hAOGEN were measured by in vitro enzyme-kinetic assays specifically developed and validated for this purpose. hPRC was determined at pH 7.4. hPRA was determined at pH 5.7 and remikren at pH 6.3. To provide excess homologous substrate during hPRA determinations, pooled plasma from 48-hour bilaterally nephrectomized TGR(hAOGEN)1623 was used containing 264 nmol/mL hAOGEN. A plasma pool from 48-hour nephrectomized Sprague-Dawley rats containing 44 nmol/mL rAOGEN was used during determinations of rPRA. Both pools had been checked for the absence of residual PRA. Human plasma prorenin concentrations were determined by a similar protocol. First, 100 μL plasma was treated with 0.1 mg bovine trypsin (200 IU/mg, Worthington No. 3744) at 4°C to activate all plasma prorenin to renin. The reaction was stopped after 90 minutes by addition of soybean trypsin inhibitor (Serva) to the incubation mixture at a concentration of 2 U per unit trypsin. Total PRA was then assayed as for hPRC. Human plasma prorenin concentration was calculated as the difference between hPRA and total renin concentration. All in vitro incubations were repeated in the presence of remikren (2.5×10−7 mol/L) to control for species specificity of the Ang I-forming pathway.

The Ang I generated during the various in vitro incubations was measured by direct radioimmunoassay. Activities and concentrations are expressed as nanograms Ang I per milliliter per hour or micrograms Ang I per milliliter, as necessary. All radioimmunoassay determinations were made in triplicate. Measurements were made at two different concentrations. Remikren or lisinopril did not interfere with measurements. The sensitivity of the radioimmunoassay as defined by the concentration of Ang I capable of displacing the 125I-labeled Ang I tracer by 50% was 3 to 4 pg per assay tube. A displacement of 50% of the tracer was achieved at a concentration of 21±4 pg per tube (n=11). The mean intra-assay and interassay variabilities were 9% and 13%, respectively.

**RNase Protection Assay**

Total RNA was isolated from snap-frozen tissues by an standard lithium chloride/mercaptoethanol precipitation technique. mRNAs specific for hAOGEN, rAOGEN, rat and hREN, and β-actin were then identified by RNase protection assay with an Ambion RPA III kit (Irvine Biotechnology GmbH) according to protocols suggested by the manufacturer. Antisense RNA probes were prepared by T7 polymerase transcription with cDNA fragments specific for hAOGEN and hAOGEN subcloned into pGEM3 and pGEM4 vectors as well as cDNA fragments specific for rREN, hREN, and rat β-actin subcloned into pGEM3 and pBluescript SK II+ vectors, as described previously. The protected sequences of the various probes were 132, 290, 297, 225, and 150 nucleotides in the above given order. All probes were radiolabeled with [32P]UTP to a specific activity of greater than 2×10⁶ cpm/μg.
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FIG 1. Top, Systolic pressure (mean±SD) of 45 double TGR (right) and Sprague-Dawley controls (left) at the time of death (age approximately 7 weeks). The difference was highly significant. Bottom, Frequency distribution of cardiomyocyte width (diameter) from seven double TGR and Sprague-Dawley (SD) control rats. The TGR distribution is displaced (P<.05), indicating wider cells. The increased width was further verified by l test (inset).

Histology

For conventional morphology, the heart and right kidney were removed and cut sagitally, and the tissue was fixed in 4% phosphate-buffered paraformaldehyde at room temperature. Subsequently, the tissue was dehydrated and embedded in paraffin. Sections 2 to 3 μm thick were cut with a microtome (Leitz 1512). The sections were then deparaffinized and rehydrated before staining with hematoxylin and eosin and periodic acid–Schiff for morphological examination.

Statistical Methods

Mean values with SD and linear regression parameters were calculated. Frequency distributions were tested by the Kolmogorov-Smirnov test. Differences between contrasting groups were tested either by one-way ANOVA or by Student’s t test using StatView software on a Macintosh computer. A value of P<.05 was regarded as significant. The terms increased and decreased are used only when significant.

Results

Data from heterozygous TGR(hREN) before and after 14 days of lisinopril treatment are given in the Table. rPRC and hPRC both increased dramatically with lisinopril more than 10-fold. We also measured human prorenin, which did not change significantly. These data indicate that the transgene of the heterozygous TGR(hREN) responds promptly and appropriately to angiotensin-converting enzyme inhibition. The double TGR values are also shown in the Table. The measurements were obtained at death and are without any treatment. hPRC was substantially higher, nearly 10-fold, in the double TGR than in the TGR(hREN). PRA was 30- to 50-fold higher than in nontransgenic control rats. There was no significant difference between male and female TGR. A linear correlation between PRA and hPRC (r=.94, P<.05) was observed. hAOGEN values were elevated to a degree similar to that in TGR(hAOGEN). Plasma hAOGEN concentrations and systolic pressure (r=.35, P=.05) were correlated in our rats, although the correlation was not robust. The rAOGEN values were similar to those of nontransgenic control rats.
Blood pressure values are shown in Fig 1 (top). The rats averaged 7 weeks of age at the time of measurement. The systolic values approached 200 mm Hg and were 80 mm Hg higher than those in control rats. There was no significant blood pressure difference between male and female double TGR. Since we euthanized the rats dependent upon their condition, we are able to comment on their survival. The youngest double TGR was euthanized at 29 days, the oldest at 81 days (mean, 55±10). Nine rats were found dead and were not included in the study. Unfortunately, we were not able to determine a histological cause of death. No evidence of stroke was found.

The hearts of double TGR averaged 0.36±0.06 g/100 g body wt, and that of control rats averaged 0.31±0.02 g/100 g body wt (P<.05). The left ventricles were visibly enlarged. At 8 weeks, the body weights of double TGR averaged 218±33 g for males and 160±15 g for females, which was not significantly different from weights of control rats. Double TGR drank 61±12 mL/d, and control rats drank 32±7 mL/d (P<.05). Fig 1 (bottom) shows a quantification of cardiomyocyte diameter. Frequency distributions of double TGR and control rats are shown (n=7); they were significantly different. The mean diameter of double TGR muscle cells was about 20% wider than that of control cells.

Histological sections from kidneys are shown in Fig 2. The glomeruli were preserved (Fig 2a and 2b); however, the vessels showed increased intimal and medial thickness as well as hyaline deposits (arrows); the tubules were frequently filled with proteinaceous material, and the interstitium showed moderate infiltration. A control section with normal small arterioles without hyaline deposits (arrowheads) from a nontransgenic Sprague-Dawley rat is shown in panel c. Periodic acid-Schiff staining. The bar indicates 50 μm.

Fig 2. Periodic acid-Schiff-stained section (a and b) from a double TGR kidney. Glomeruli were preserved; however, vessels showed increased intimal and medial thickness as well as hyaline deposits (arrows); the tubules were frequently filled with proteinaceous material; and the interstitium showed moderate infiltration. A control section with normal small arterioles without hyaline deposits (arrowheads) from a nontransgenic Sprague-Dawley rat is shown in panel c. Periodic acid-Schiff staining. The bar indicates 50 μm.

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Fig 3. Section from a normal heart (A) and from a double TGR heart (B) stained with hematoxylin/eosin. Individual cardiomyocytes were significantly larger in cross section in double TGR. Remarkably little interstitial fibrosis was present. Bar indicates 50 μm.
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Fig 4A shows an RNase protection assay of 13 different tissues for hREN gene expression in a heterozygous TGR(hREN). Expression was detected in aorta, adrenal gland, ovary, skeletal muscle, spleen, kidney, and lung. None was found in brain or liver under these conditions. The double TGR (Fig 4B) showed rREN and hREN. rREN was found in kidney, with a trace in the adrenal gland. hREN was found in the same tissues as in the heterozygous rat. rAOGEN (Fig 4C) was found in liver, brain, and cerebellum. hAOGEN (Fig 4C) was found in all organs at different levels. The liver and kidney showed the highest values.

Lisinopril administration in six double TGR decreased blood pressure from 191±25 mm Hg on day 0 to 129±7 mm Hg by day 7 (P<.05). Remikiren decreased blood pressure from 190±24 mm Hg on day 0 to 124±16 mm Hg by day 7 (n=6, P<.05). The blood pressure decreases were not different for the two compounds.

Discussion

The important findings in this study were that a TGR(hAOGEN) and TGR(hREN) cross produced offspring with chronic, severe, high hREN hypertension in the rat. Blood pressure values were markedly elevated, severe cardiac hypertrophy resulted, the kidneys developed nephrosclerosis even with both organs in place, and the rats died untreated after about 8 weeks. Circulating hPRC and hAOGEN were detected at high levels, although there was considerable variability in the values, which appeared to be greater than the variability in blood pressure. The hAOGEN transgene was expressed in all tissues tested but was highest in the liver. The hREN transgene was present in kidney but not in liver. An hREN inhibitor and an angiotensin-converting enzyme inhibitor both reduced the blood pressure to normal values, documenting the mechanism of the blood pressure elevation.

Ours is not the first double transgenic model of high hREN hypertension. Fukamizu et al11 developed double transgenic mice harboring both the hAOGEN and hREN genes. They were able to demonstrate a sustained increase in blood pressure in crossmated separate lines and showed that the hypertension in the hybrid mice was initiated by the interaction between the products of the two human genes. They proved their point by lowering blood pressure in hypertensive mice with an angiotensin-converting enzyme inhibitor, a Ang II receptor blocker, and an hREN-specific renin inhibitor similar to the one we used. Merrill et al12 also generated transgenic mice harboring both the hAOGEN and hREN genes. hPRA and Ang II levels were both elevated in their model, and the mice were hypertensive with mean systolic values of 170 mm Hg. The authors observed a resetting of the baroreflex, without alterations in baroreflex sensitivity. Our double transgenic rats are understandably quite similar to the double transgenic mice of Fukamizu et al and Merrill et al. They observed hREN and hAOGEN transgene expression in the kidneys, whereas only hAOGEN was expressed in liver. We observed similar transgene expression in the rat.

Our experiment demonstrates once again the species specificity of the renin-angiotensin system.13-16 hREN does not
not proteolytically cleave rat rAOGEN effectively and neither does rREN cleave hAOGEN. Thus, both parental transgenic lines were normotensive. The TGR(hREN) had higher hPRC than rPRC values and, interestingly, had substantial levels of human prorenin hPRC and rPRC both increased significantly after lisinopril, indicating that the hREN transgene is physiologically regulated. In the double transgenic cross, the hPRC values were similar to those in the parental strain, despite the elevated blood pressure. The hAOGEN values were somewhat lower than those we reported earlier for the parental strain. These measurements varied substantially, and refinements in breeding of subsequent lines will be necessary. We observed a significant correlation between hAOGEN and systolic pressure in the double TGR cross but could find none between hREN and blood pressure.

The transgene expressions we observed in our double TGR cross were similar to those reported earlier for mice, namely, hREN was expressed predominantly in the kidney and hAOGEN was most prominent in liver, although in our study we found hAOGEN to be widely distributed. The distribution of the transgenes is consistent with the promoters used. For hREN, the entire gene was used, including its own promoter region. In the case of hAOGEN, the endogenous promoter was also used, explaining the high expression in liver tissue. The hREN expression is different from the rREN expression described by Ohkubo et al., who found the rat transgene to be predominantly expressed in the liver of transgenic mice. They used transgenes consisting of a fusion between the metallothionein promoter and the coding region of either renin or angiotensinogen. This approach resulted in unregulated expression of rREN and rAOGEN mRNA in the liver, the major site of metallothionein promoter activity. In their study, Ohkubo et al found that mice harboring the rREN gene became severely hypertensive. We observed earlier that rats harboring a mouse renin (mREN2) gene also became severely hypertensive. Furthermore, mice carrying the rAOGEN gene are hypertensive as well.

In the present study, we examined the hearts and kidneys morphologically. The hearts of rats carrying both transgenes were remarkably hypertrophied, which we were able to document not only with organ weight but also by quantitative measurement of the cardiomyocytes. Both pressure overload and Ang II acting directly serve to increase left ventricular mass without affecting myocardial isofrom mRNAs. We also found that vascular disease and nephrosclerosis were present in our rats. Ang II has been shown to promote intercellular adhesion molecule-1 and collagen expression. Furthermore, Ang II also leads to transforming growth factor-β gene expression. The double transgenic strain we have developed will facilitate research on the mechanisms of cardiac and renal damage secondary to pressure overload as well as Ang II-dependent mechanisms.

Although many physiological observations can now be conducted in mice, rats still have the advantage of size, permitting more detailed assessments of renal and cardiac functions. We have reported a transgenic rat model in which lifelong overexpression of both hREN and hAOGEN lead to severe, sustained hypertension with end-organ, notably cardiac and renal, damage. The model is unique in that it will allow study of Ang II–related mechanisms in a system that can easily be evaluated physiologically. Genetic and physiological manipulations can be conducted simultaneously. Moreover, the model relies on a human renin-angiotensin system components. Thus, certain pharmacological investigations of species-specific agents such as hREN inhibitors become possible in a rat model. We have presented preliminary data documenting this feature.

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