Genetic Susceptibility to Hypertension-induced Renal Damage in the Rat

Evidence Based on Kidney-specific Genome Transfer

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Abstract

To test the hypothesis that genetic factors can determine susceptibility to hypertension-induced renal damage, we derived an experimental animal model in which two genetically different yet histocompatible kidneys are chronically and simultaneously exposed to the same blood pressure profile and metabolic environment within the same host. Kidneys from normotensive Brown Norway rats were transplanted into unilaterally nephrectomized spontaneously hypertensive rats (SHR-RT1.N strain) that harbor the major histocompatibility complex of the Brown Norway strain. 25 d after the induction of severe hypertension with deoxycorticosterone acetate and salt, proteinuria, impaired glomerular filtration rate, and extensive vascular and glomerular injury were observed in the Brown Norway donor kidneys, but not in the SHR-RT1.N kidneys. Control experiments demonstrated that the strain differences in kidney damage could not be attributed to effects of transplantation-induced renal injury, immunologic rejection phenomena, or preexisting strain differences in blood pressure. These studies (*a*) demonstrate that the kidney of the normotensive Brown Norway rat is inherently much more susceptible to hypertension-induced damage than is the kidney of the spontaneously hypertensive rat, and (*b*) establish the feasibility of using organ-specific genome transplants to map genes expressed in the kidney that determine susceptibility to hypertension-induced renal injury in the rat. (*J. Clin. Invest.* 1997. 100: 1373–1382.) Key words: glomerulosclerosis • nephrosclerosis • genetics • kidney transplantation • congenic rat strains

Introduction

One of the key questions in hypertension research is why nephrosclerosis occurs in some patients with high blood pressure, but not in others. Although the extent to which mild to moderate hypertension can initiate renal damage is unclear, it is well-established that malignant hypertension can induce renal failure and that mild to moderate hypertension can accelerate the progression of renal disease (1–4). A number of environmental, disease, and treatment variables may affect the risk for hypertension-induced or accelerated renal disease. It is also widely believed, however, that genetic factors play an important role in susceptibility to hypertension-induced or accelerated renal damage (5–7). In humans, the evidence that genetic factors might affect susceptibility to hypertension-induced or accelerated renal injury is based largely on the finding of different rates of hypertension-associated kidney disease in individuals from different ethnic groups presumed to have been exposed to comparable degrees of hypertension for similar periods of time (5–7). In animal models, genetic factors are also suspected to affect susceptibility to hypertension-induced or accelerated renal disease (7–11).

In a strict sense, differences in the degree or frequency of renal damage associated with high blood pressure cannot be traced to differences in genetic susceptibility to hypertension-induced or accelerated renal injury unless one can rigorously control for the chronic blood pressure load on the kidneys as well as for the many other factors that can interact with hypertension to promote renal damage (e.g., dietary factors, hyperlipidemia, hyperglycemia, etc.). Moreover, it can often be difficult to discern whether hypertension is a determinant of renal damage or a secondary consequence of intrinsic renal disease. Given the complex nature of the pathogenesis of target organ damage in hypertension and the difficulty of adjusting for the multiple variables that can influence renal function and blood pressure, the ultimate goal of mapping specific quantitative trait loci (QTLs)[1] that determine susceptibility to hypertension-induced or hypertension-accelerated renal injury remains a daunting task.

Most studies designed to investigate the role of genetic factors in susceptibility to hypertension-induced renal disease have typically involved a small number of blood pressure measurements obtained with indirect methods over relatively short periods of time. In humans (12) and in rats (13, 14), blood pressure fluctuates rapidly and undergoes diurnal variations, and there is evidence to suggest that both the amplitude and the frequency of the blood pressure fluctuations independently contribute to the risk for target organ damage (12–14). To date, no study has actually identified individuals with different

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1. *Abbreviations used in this paper:* BN rat, Brown Norway rat; DOCA, deoxycorticosterone acetate; H&E, hematoxylin and eosin; PAS, periodic acid Schiff; QTL, quantitative trait loci; SHR, spontaneously hypertensive rat.

degrees or rates of hypertension-induced renal damage who have been chronically exposed to exactly the same blood pressure load within the same environment. Moreover, many studies have not distinguished between the condition of renal damage induced or accelerated by hypertension versus the condition of hypertension secondary to renal damage.

To test the hypothesis that genetic factors can determine susceptibility to hypertension-induced renal damage, we have applied state-of-the-art kidney transplantation techniques in a congenic strain of spontaneously hypertensive rat (SHR) to derive a novel experimental model in which two genetically different yet histocompatible kidneys are chronically and simultaneously exposed to the same blood pressure profile and metabolic environment within the same host. In this model, we (*a*) demonstrate that the kidney of the normotensive Brown Norway (BN) rat is inherently much more susceptible to hypertension-induced damage than is the kidney of the SHR, and (*b*) have established the feasibility of using organ-specific genome transplants to begin mapping QTLs expressed in the kidney that determine susceptibility to hypertension-induced renal damage in the rat.

Methods

Animals and animal care. Rats of the SHR-RT1.N congenic strain (previously referred to as SHR.1N [15]) were obtained from a colony maintained at the University of California, San Francisco, and served as histocompatible recipients of kidneys from normotensive BN donors. The SHR-RT1.N congenic strain was originally derived at the Czech Academy of Sciences in Prague (15) by transferring a piece of chromosome 20 containing the MHC of the BN strain onto the genetic background of a progenitor strain of SHR (SHR/Ola) that descended from the SHR colony at the National Institutes of Health. This transfer was accomplished using a backcross breeding and immunogenetic phenotyping protocol in which the MHC of the SHR (RT1.K haplotype) was replaced with the MHC of the BN-Lx rat (RT1.N haplotype). SHR-RT1.N animals of the N14F22 generation were used in this study. Based on the number of backcross generations and a preliminary assessment of molecular markers within and flanking the MHC (Tnfa, Hspa1, D20Arb548, D20Arn249, Prkacn2), the size of the differential chromosome segment in the SHR-RT1.N congenic strain is estimated to be $\sim$ 31 cM.

BN rats were obtained from two different commercial suppliers; Harlan Sprague Dawley Inc. (Indianapolis, IN) and Charles River Laboratories (Wilmington, MA), to insure that the results were not dependent on the source of the BN donor kidneys used in the transplantation experiments. SHR from Harlan Sprague Dawley Inc. were also used in some experiments to confirm that the SHR-RT1.N congenic strain was not uniquely resistant to hypertension-induced renal injury. All rats were cared for in accordance with the Principles of the Guide for the Care and Use of Laboratory Animals (Department of Health, Education, and Welfare). They were housed in a constant-temperature room with a 12-h light and 12-h dark cycle, and they had free access to tap water with or without additional salt, and to Purina Rodent Chow, except that food was restricted before surgery.

Experimental protocols. The basic experimental protocol involved transplantation of kidneys into unilaterally nephrectomized recipients. This design allowed for measurement of blood pressure and renal function, and assessment of renal histology in rats with one transplanted kidney and one native kidney. To accelerate the development of hypertension and renal injury as described by Sesoko et al. (16), the rats were treated with deoxycorticosterone acetate (DOCA, by subcutaneous pellet, 100 mg/kg body wt), and were given a salt solution (1% NaCl and 0.2% KCl) to drink. Four groups of rats were studied. In group 1, kidneys from BN donors (five females and one male) were transplanted into BN recipients (five females and one male, respectively). This group served to demonstrate that the transplant procedure per se did not damage BN donor kidneys. We also found, however, that BN rats were completely resistant to the development of DOCA-salt hypertension, and therefore, all subsequent studies were performed using SHR or SHR-RT1.N rats as recipients. In group 2, kidneys from SHR or SHR-RT1.N donors were transplanted into SHR or SHR-RT1.N recipients, respectively. This group also was studied to insure that the transplant procedure did not damage donor kidneys or predispose them to hypertension-induced renal injury. These experiments were performed in five males and three females. In group 3, kidneys from BN donors (five females and three males) were transplanted into histocompatible SHR-RT1.N recipients (five females and three males, respectively). This group enabled us to compare susceptibility of BN versus SHR-RT1.N kidneys within the same hypertensive and metabolic environment. In group 4, kidneys from BN donors (six males) were transplanted into SHR-RT1.N recipients (six males) that had been pretreated with triple antihypertensive therapy (200 mg hydralazine + 50 mg hydrochlorothiazide + 5 mg reserpine per liter of drinking water, as described by Bidani et al. [17]) from weaning at 20–25 d of age. This treatment rendered the SHR-RT1.N recipients normotensive before transplantation, and enabled us to test whether the relative resistance of SHR-RT1.N kidneys to hypertension-induced damage was dependent on prior exposure and adaptation of the SHR-RT1.N kidneys to hypertension.

Kidney transplantation. Donors and recipients were age-matched (6–8 wk old at the time of transplantation unless stated otherwise), and the recipients were unilaterally nephrectomized. The techniques for harvesting and transplanting kidneys were described in detail previously (18). In brief, donors and recipients were anesthetized and maintained on a surgical plane of anesthesia with Na pentobarbital (initial dose $\sim$ 45 mg/kg body wt, given via tail vein). The donor rat was heparinized (100 mU in 0.1 ml, i.v.) and the left kidney was removed after it was flushed via the aorta with 5 ml of an ice-cold solution (150 mM NaCl and 200 mM mannitol, pH 6.4). The donor kidney was kept in ice-cold flush solution while preparing the recipient: a midline incision was made, and the left kidney was removed after transecting the ureter near the hilum, the renal artery near its origin (or distal to the inferior adrenal artery if it arose from the renal artery rather than the aorta), and the renal vein near the kidney, leaving the adrenal and spermatic/ovarian veins patent. With this method, the circulation of both of the recipient's adrenal glands remained intact. The donor kidney was placed on an ice-cold cooling coil during anastomosis of the vessels; end-to-side of the donor kidney's artery and the recipient's aorta, and end-to-end of the donor kidney's and the recipient's renal veins. The vessels were unclamped, and the cooling coil was removed. After performing an end-to-end anastomosis of the recipient's and the donor kidney's ureters, the abdominal wall was closed with a continuous 6-0 prolene suture, the skin was closed with interrupted 6-0 silk sutures, and the rat was put in a recovery cage with access to food and water.

Blood pressure monitoring and induction of DOCA-salt hypertension. 7 to 14 d after transplant, the recipients were anesthetized (Na pentobarbital, $\sim$ 45 mg/kg body wt given via tail vein) and instrumented for continuous blood pressure monitoring by radiotelemetry (13). After a 3–5-d recovery period, the recipients were housed individually in polycarbonate cages placed on radioreceivers connected to a 486 microcomputer running the Dataquest Software Package (Data Sciences International, Akron, OH). Pulsatile arterial blood pressure and heart rate were recorded for a 5-s interval every 10 min throughout the day and night for 1–2 wk. Then, except for two rats in group 4, each rat was anesthetized (sodium pentobarbital; $\sim$ 45 mg/kg body wt given via tail vein), and a sustained delivery DOCA pellet (Innovative Research; Sarasota, FL) was implanted subcutaneously. After recovery, salt (1% NaCl + 0.2% KCl) was added to the drinking water, and blood pressure monitoring was continued for 25 d.

Clearance studies. After 25 d of DOCA-salt treatment, split renal function studies were performed using previously described clearance

techniques (13). In brief, rats were anesthetized and maintained on a surgical plane of anesthesia with Na pentobarbital (initial dose ~ 45 mg/kg body wt given via tail vein). The trachea was isolated and cut to facilitate spontaneous respiration, and a femoral vein and artery were cannulated for intravenous infusion of solutions, and for sampling arterial blood and measuring arterial blood pressure with a pressure transducer connected to a polygraph. A midline abdominal incision was made, and both ureters were cannulated. Then, a priming injection of inulin was given via the femoral vein catheter (2 ml per kg body wt of 10 g % inulin dissolved in 150 mM NaCl). This injection was followed by a continuous intravenous infusion of inulin (0.1 ml per min of 1.9 g % inulin dissolved in 150 mM NaCl). After a 1-h equilibration period, two consecutive 30-min clearance periods were begun. Urine was collected separately from each kidney into preweighed micro test tubes, and arterial blood samples were collected at the clearance midpoints. The blood was centrifuged, and the plasma and urine were frozen until analyzed as described below.

Histologic studies. At the end of the second clearance period, the kidneys were perfusion-fixed at the ambient pressure as described previously (17). In brief, the kidneys were perfused with 150 mM NaCl at 38°C until the venous effluent cleared, followed by modified Karnovsky's fixative (2% wt/vol paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4) for 10 min. Two transverse sections of the kidney through the papilla were post-fixed in buffered formalin and embedded in paraffin. Sections (3–4 μm) were stained with hematoxyln and eosin (H&E) and periodic acid Schiff (PAS). Glomerular and vascular injury were quantitated separately in both of the sections from each kidney as previously described (17). All of the glomeruli in each section were counted and classified as normal or abnormal. Abnormal glomeruli were separated into those exhibiting (*a*) acute hypertensive injury (necrosis, thrombosis, microembolisms and capillary wall disruption); (*b*) segmental glomerular sclerosis (collapsed capillary loops with mesangial matrix expansion); and (*c*) ischemic injury (globally shrunk glomeruli with collapsed capillary loops). The percentages of glomeruli exhibiting each of these three lesions were recorded. The total number of vascular profiles exhibiting evidence of acute hypertensive injury (fibrinoid necrosis, myointimal proliferation, fragmentation of internal elastic lamellae, and aneurysmal dilatation) was counted in each section. The number of such vascular profiles with injury was expressed per 100 glomeruli as a vascular injury score to correct for any differences in the amount of renal parenchyma present in sections from individual kidneys.

Analyses, calculations, and statistics. Inulin concentrations in urine and plasma filtrates, and protein concentration in urine, were determined by spectrophotometry (17). Inulin clearance was calculated and taken as the GFR. Protein excretion (μg per min per ml GFR) was calculated and taken as an index of the integrity of the glomerular capillary barrier. Renal function values obtained during the two clearance periods were averaged. All results (blood pressure, clearance, and histology) are expressed as means±SEMs. As appropriate, paired *t* tests and ANOVA with Scheffe contrasts were used to assess the statistical significance of differences in means (19). P values < 0.05 were considered significant.

Results

Group 1. In BN recipients of BN donor kidneys, we determined the effects of DOCA-salt treatment on blood pressure, and investigated whether the transplant procedure itself impaired renal function or structure. As shown in Fig. 1 *A*, the BN rats were remarkably resistant to the effects of DOCA-salt treatment on blood pressure. In fact, the overall averages of systolic blood pressure before and during administration of DOCA-salt were virtually identical: 126±3 versus 127±2 mmHg, respectively. Because it was not possible to induce DOCA-salt hypertension in BN rats, we could not use BN recipients to investigate the susceptibility of transplanted SHR versus native BN kidneys to hypertension-induced damage. In fact, administration of DOCA-salt failed to increase blood pressure, even in unilaterally nephrectomized BN rats. Therefore, sub-

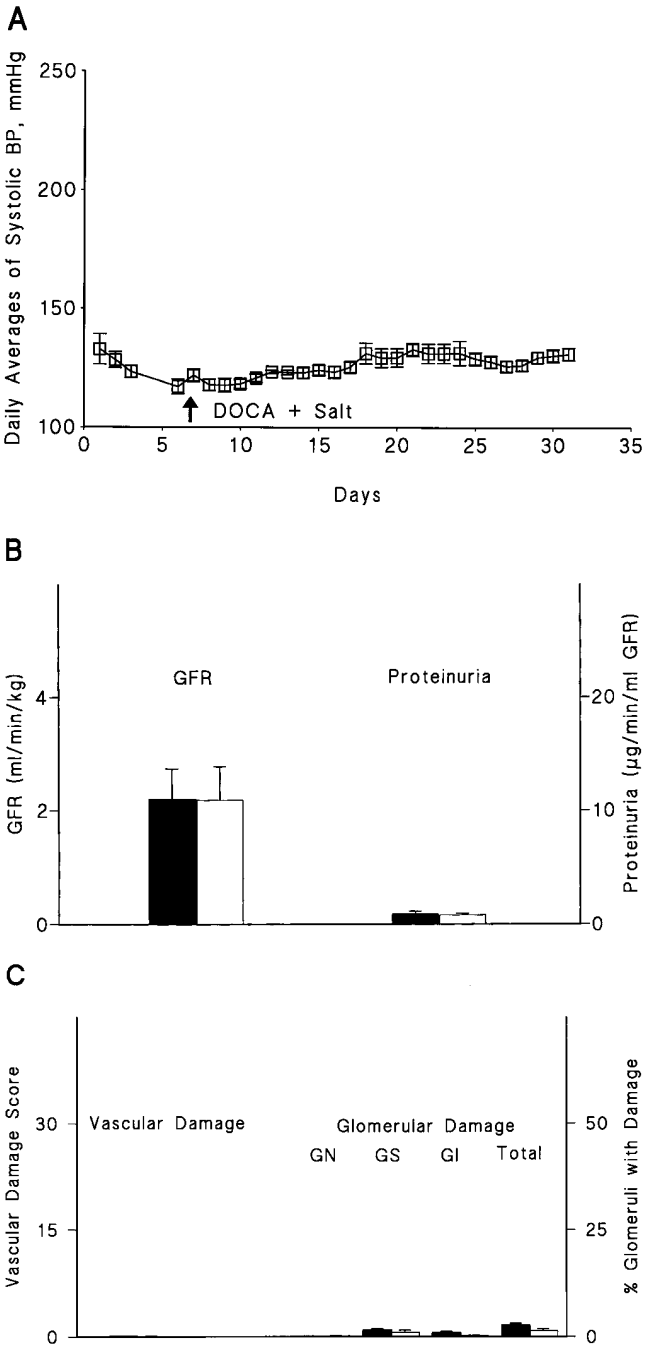


Figure 1. Blood pressure, renal function, and renal histology in unilaterally nephrectomized BN recipients of BN donor kidneys (group 1; $n = 6$, five females and one male). (*A*) Daily averages of systolic blood pressure before and during administration of DOCA-salt. In each rat, systolic blood pressure was sampled for 5 s every 10 min, 24 h a day. (*B*) GFRs and protein excretion rates of transplanted and contralateral native BN kidneys. (*C*) Vascular and glomerular damage scores in transplanted and contralateral native BN kidneys. GN, glomerular necrosis; GS, glomerular sclerosis; GI, glomerular ischemia; total, sum of the three individual glomerular damage scores. See text for explanation of damage scoring. *Black bars*, transplanted BN kidney; *white bars*, native BN kidney.

sequent studies were performed using DOCA-salt–treated SHR or SHR-RT1.N rats as recipients.

Although it was not possible to induce DOCA-salt hypertension in the BN recipients of BN donor kidneys, this group could be used to study the effects of the transplant procedure itself on the function and morphology of BN donor kidneys. Studies of bilateral renal function and morphology showed no differences between the transplanted BN kidneys and the contralateral native BN kidneys. The GFRs of the transplanted kidneys, 2.2±0.5 ml/min per kg body wt were identical to the GFRs of the contralateral native kidneys, 2.2±0.6 ml/min per kg body wt (Fig. 1 *B*). In the transplanted and native kidneys, rates of protein excretion were 0.9±0.3 and 0.8±0.1 μg/min per ml GFR, respectively. Moreover, there was no histologic evidence of damage in the transplanted kidneys; the vascular and glomerular damage scores of the transplanted kidneys were identical to those of the contralateral native kidneys (Fig. 1 *C*). These findings demonstrate that BN kidneys are not unusually susceptible to transplant-induced damage.

Group 2. In SHR (or SHR-RT1.N) recipients of SHR (or SHR-RT1.N) donor kidneys, we found that the transplant procedure did not damage the transplanted kidneys or predispose them to hypertension-induced injury. Fig. 2 *A* shows the daily averages of systolic blood pressure in these transplant recipients before and during administration of DOCA-salt. The overall averages of systolic blood pressure before and during administration of DOCA-salt were 162±2.8 and 198±5.8 mmHg, respectively ($P < 0.0002$).

After 25 d of DOCA-salt treatment, the GFRs and protein excretion rates of the transplanted and therefore denervated kidneys (2.7±0.21 ml/min per kg body wt, and 0.9±0.3 μg/min per ml GFR, respectively) were nearly the same as those of the contralateral native kidneys with intact nerves (2.5±0.4 ml/min per kg body wt, and 0.5±0.1 μg/min per ml GFR, respectively) (Fig 2 *B*). These results indicate that despite exposure to severe hypertension, renal function in transplanted and therefore denervated kidneys was similar to that in the contralateral native kidneys with intact renal nerves.

The average histologic damage scores for both transplanted and native kidneys are shown in Fig. 2 *C*. The transplanted and native kidneys were not significantly different with respect to any of these damage scores, which also indicates that transplanting a kidney does not increase its susceptibility to hypertension-induced damage (note: the results obtained when transplanting a kidney from an SHR-RT1.N donor into an SHR-RT1.N recipient were similar to those obtained when transplanting a kidney from a Harlan SHR donor into a Harlan SHR recipient). In addition, the results of the three female and five male recipients were combined because no sexual dimorphism was obvious with respect to either blood pressure or hypertensive damage. It is possible, however, that with larger groups of males and females, differences might be found. In some genetic models of hypertension including SHR, blood pressure is higher in males than in females (20). In DOCA-salt induced hypertension, however, the literature is contradictory, with one report of no differences in blood pressure between male and female rats (21), and another report of higher blood pressures in male than in female rats (22). Similarly, although male rats are more susceptible to some forms of renal injury than are female rats (8), the literature on DOCA-salt–induced renal injury again is contradictory, with one report that male and female rats are equally susceptible (23), and another report that female rats are more susceptible than males (24).

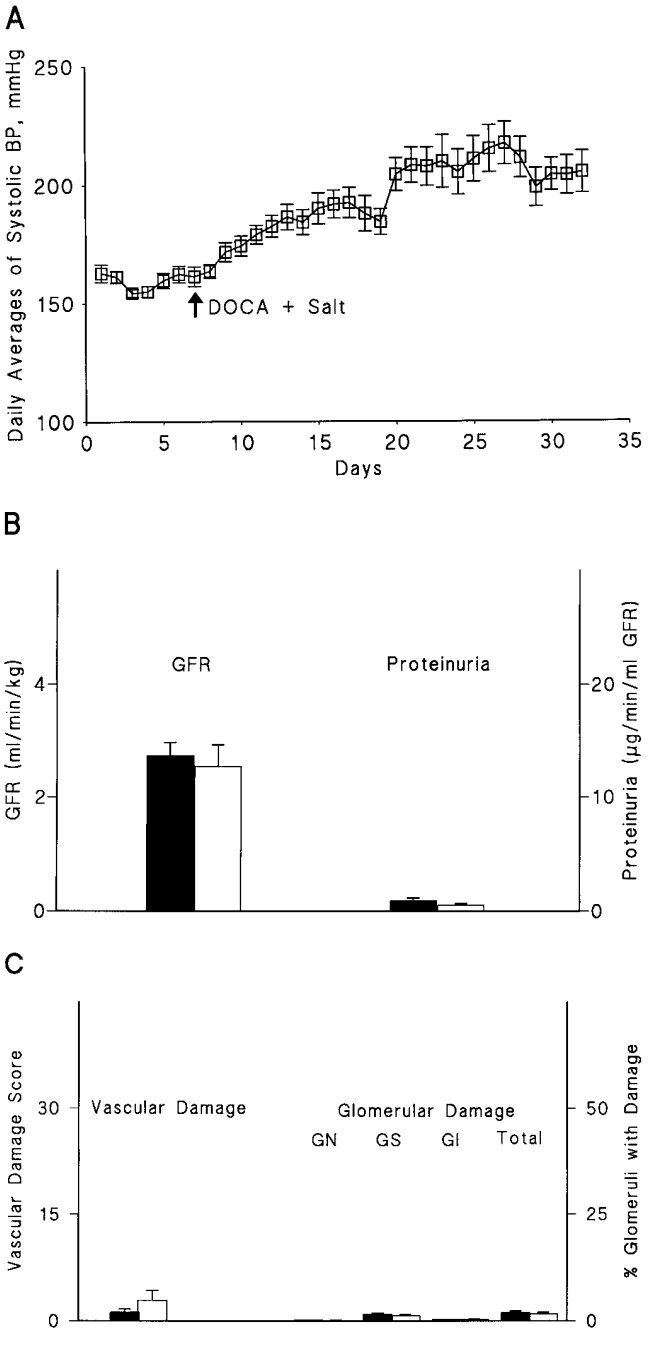


Figure 2. Blood pressure, renal function, and renal histology in unilaterally nephrectomized SHR (or SHR-RT1.N) recipients of SHR (or SHR-RT1.N) donor kidneys (group 2; $n = 8$, five males and three females). The results obtained from SHR and SHR-RT1.N rats have been combined. See legend to Fig. 1 for panel descriptions and abbreviations. *Black bars*, transplanted SHR kidney; *white bars*, native SHR kidney.

Group 3. To investigate susceptibility of BN kidneys to hypertension-induced damage, we studied DOCA-salt treated SHR-RT1.N rats with transplanted kidneys from histocompatible BN donors. Fig. 3 *A* shows the systolic blood pressure of SHR-RT1.N recipients of BN donor kidneys before and dur-

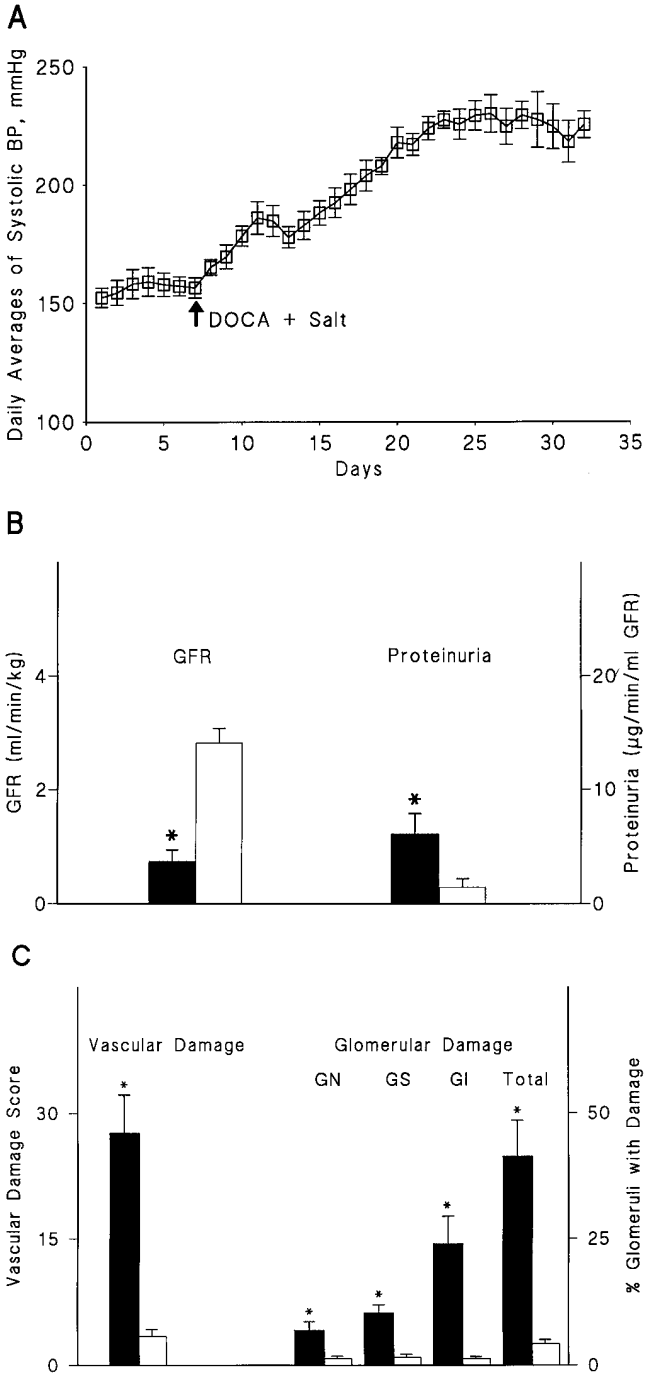


Figure 3. Blood pressure, renal function, and renal histology in unilaterally nephrectomized SHR-RT1.N recipients of BN donor kidneys (group 3; $n = 8$, five females and three males). See legend to Fig. 1 for panel descriptions and abbreviations. *$P < 0.02$ maximum, BN kidney versus SHR-RT1.N kidney. *Black bars*, BN kidney; *white bars*, SHR-RT1.N kidney.

ing DOCA-salt treatment. The overall averages of systolic blood pressure in all eight recipients were 156±4.1 and 202±3.5 mmHg before and during DOCA-salt ($P < 0.0001$). After 25 d of DOCA-salt treatment, BN donor kidneys had significantly lower GFRs than did SHR-RT1.N native kidneys (BN versus SHR-RT1.N, 0.7±0.2 versus 2.8±0.3 ml/min per kg body wt, $P < 0.001$) (Fig. 3 *B*). Also, BN kidneys had higher protein excretion rates than did SHR-RT1.N kidneys (BN versus SHR-RT1.N, 6.1±1.8 versus 1.4±0.8 μg/min per ml GFR, $P < 0.02$) (Fig. 3 *B*). These data demonstrate greater functional damage in BN kidneys than in SHR-RT1.N kidneys.

Fig. 4 illustrates the typical and contrasting histology observed in the transplanted BN and the native SHR-RT1.N kidneys in these rats. Severe hypertensive damage was observed in the BN kidneys. In contrast, the SHR-RT1.N kidneys exhibited minimal histologic injury despite being exposed to the same degree of hypertension. The histologic pattern of damage was characteristic of malignant nephrosclerosis. Vascular lesions of fibrinoid necrosis, myointimal proliferation (onion-skinning), aneurysmal dilatation, and thrombosis were typically seen (Fig. 5). Glomerular capillaries showed similar evidence of severe hypertensive damage in the form of lesions of fibrinoid necrosis and mesangiolysis, segmental sclerosis, and ischemia (Fig. 6). There was no tubulointerstitial pathology suggestive of graft rejection in any of these recipients, at a total of 7–8 wk after transplant.

The averages of the vascular damage scores, the three separate glomerular damage scores (glomerular necrosis, glomerulosclerosis, and glomerular ischemia), and the total glomerular damage scores (the sum of the three separate scores) for both kidneys of these recipients are shown in Fig. 3 *C*. All of these scores were 5–15-fold higher in the BN than in the SHR-RT1.N kidneys, despite exposure to exactly the same blood pressure profile and metabolic environment 24 h a day for several weeks. Thus, the BN kidney is much more susceptible to hypertensive damage than is the SHR-RT1.N kidney.

Fig. 7 shows the ratios of the vascular and total glomerular damage scores (left transplanted kidney/right native kidney) for rats in group 2 and group 3. In the SHR-RT1.N recipients of transplanted SHR-RT1.N kidneys, the ratios of vascular and glomerular damage in the transplanted kidney to those in the native kidney are ~ 1, indicating that the transplantation procedure itself did not affect susceptibility to hypertension-induced renal damage. In contrast, in the SHR-RT1.N recipients of transplanted BN kidneys, the ratios of vascular and glomerular damage in the transplanted BN kidney to those in the native kidney are ~ 10. This contrast illustrates that the BN kidneys are remarkably more susceptible to hypertension-induced damage than are the SHR-RT1.N kidneys.

Group 4. These experiments were designed to exclude another nongenetic explanation of the differential damage of SHR and BN kidneys observed in group 3. It is possible that exposing kidneys to a moderate degree of hypertension induces some form of adaptation that then protects the kidneys from the subsequent severe hypertension induced by DOCA-salt. Thus, in group 3, the native SHR kidneys, but not the transplanted BN kidneys, could have adapted to preexisting hypertension, and therefore could have been protected from the severe hypertension subsequently induced by DOCA-salt. To exclude this possibility, SHR-RT1.N recipients in group 4 were given triple antihypertensive therapy to normalize their blood pressure beginning at 20–25 d of age. At 10–11 wk of age, they were used as recipients of BN kidneys from age-matched donors. Antihypertensive therapy continued, radiotelemetric monitoring devices were implanted at 13–14 wk of age, and blood pressure was monitored for one more week. Then, triple therapy was discontinued and blood pressure was monitored for two more weeks. At the end of this time, four of

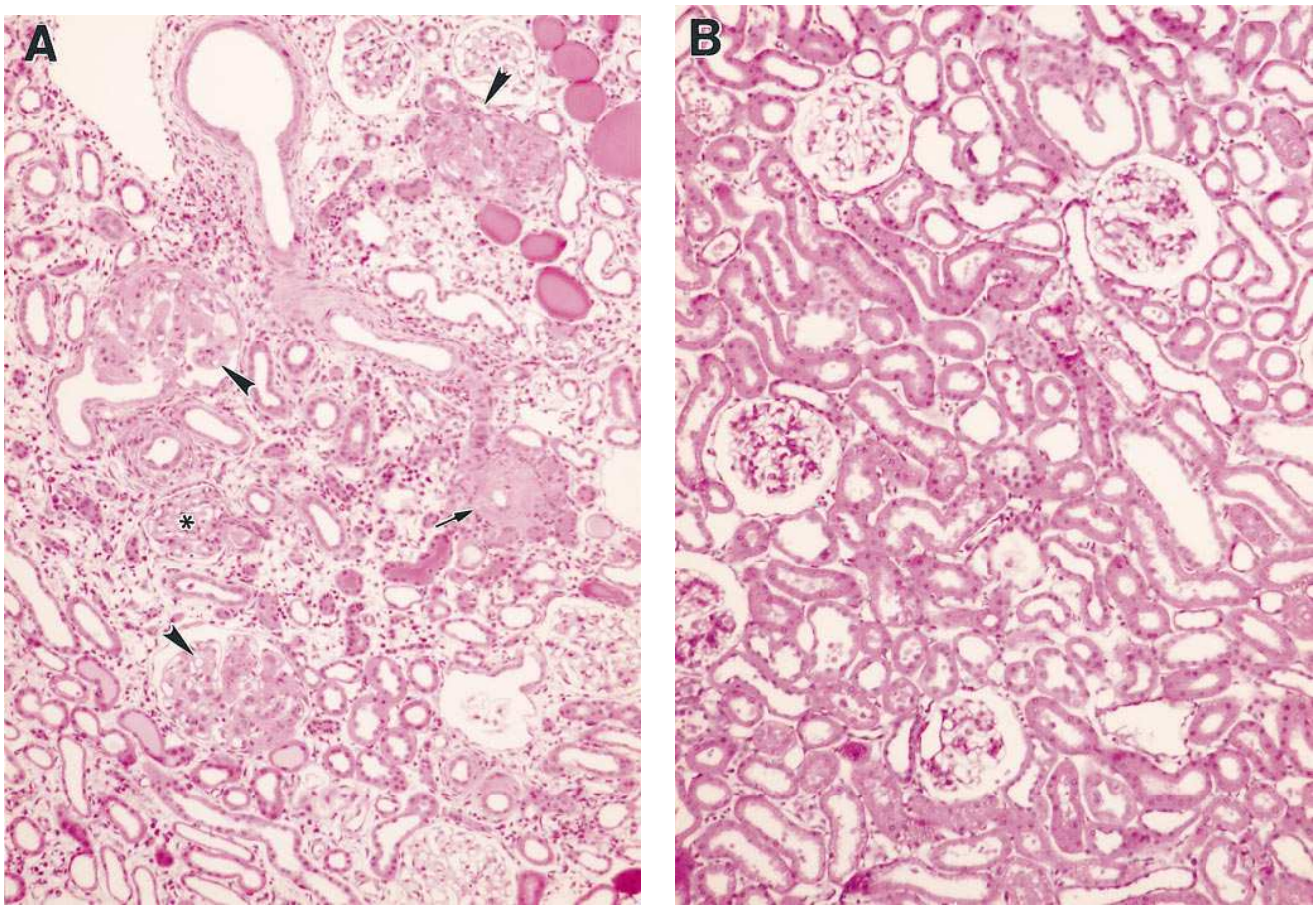
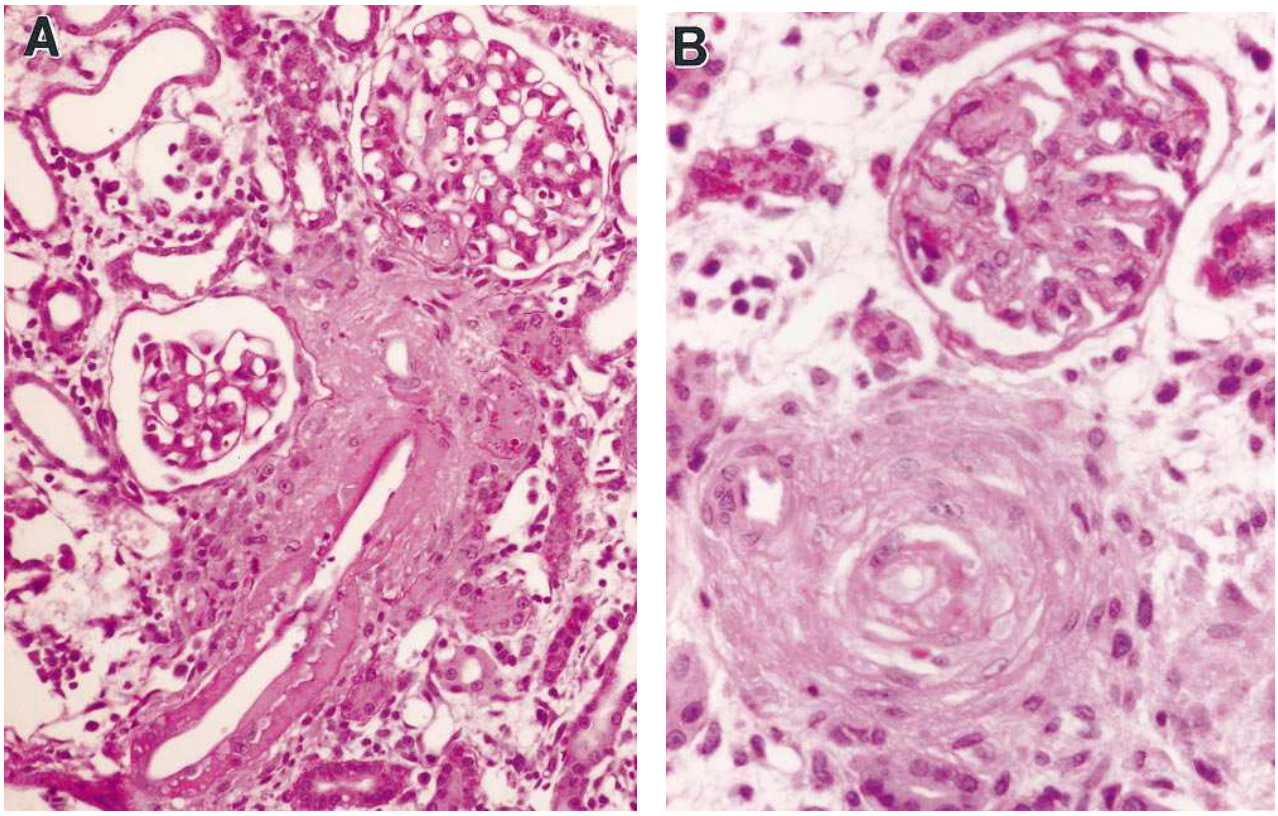


Figure 4. Survey views of the typical histological findings in the two genetically different but histocompatible kidneys in the same recipient. (*A*) BN kidney, hematoxylin and eosin (H&E), ×160; (*B*) SHR-RT1.N kidney; PAS, ×160. The striking difference between the histology of the two kidneys is readily apparent. The BN kidney shows the characteristic vascular and glomerular lesions of malignant nephrosclerosis. Segmental fibrinoid necrosis (*arrow*) of an interlobular artery is seen. Several glomeruli (*arrowheads*) show varying degrees of tuft necrosis; one glomerulus (*asterisk*) shows early ischemic change. In contrast, the SHR-RT.1N kidney shows virtually no pathology; the glomeruli are normal, the tubules are closely spaced, and the extraglomerular vessels are inconspicuous.



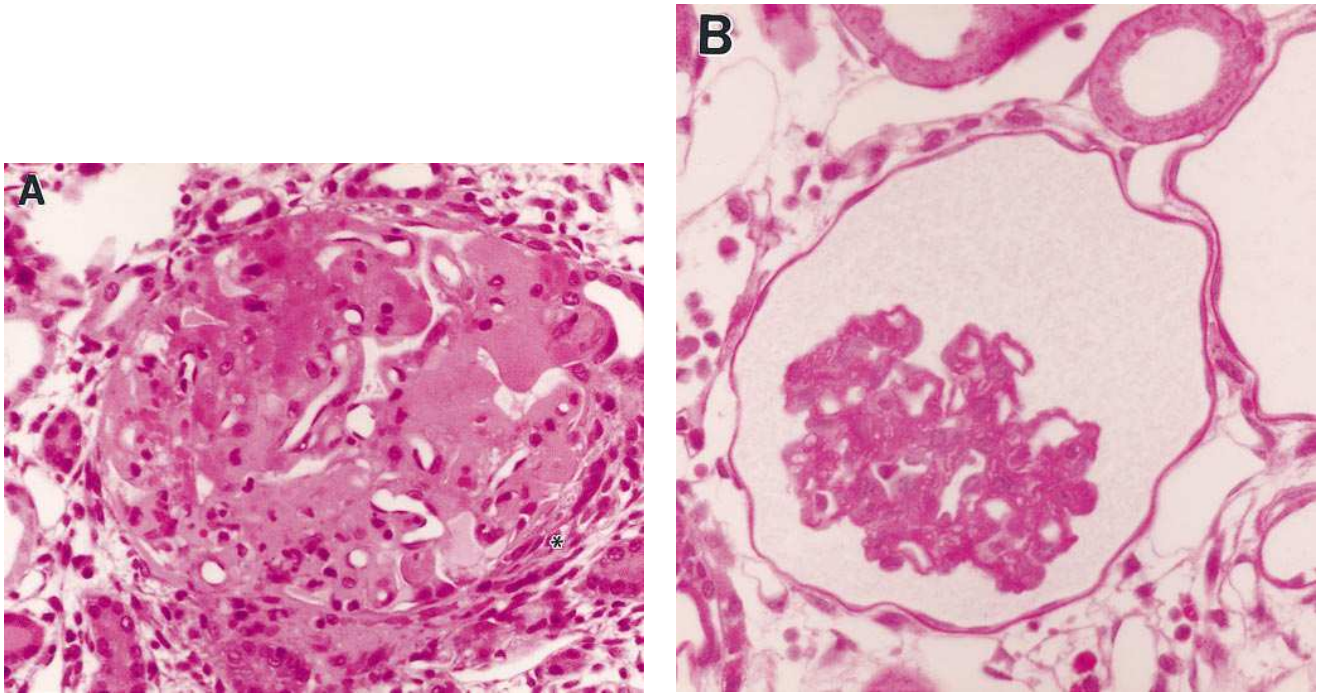


Figure 6. Spectrum of glomerular pathology observed in the BN kidneys. (*A*) Acute hypertensive injury. The glomerulus is enlarged with necrosis of the tuft and mesangiolysis. The capillaries are filled with erythrocytes and fibrin. There is also loss of endothelial cells. A small crescent is present (*asterisk*). H&E, ×600. (*B*) A glomerulus showing ischemic injury with tuft collapse and typical garland-like wrinkling and thickening of the capillary walls. There is also loss of mesangial cells and matrix. PAS, ×600.

these recipients were given DOCA-salt, and blood pressure was monitored for 25 d. Two recipients were not given DOCA-salt, and their blood pressures were monitored for an additional 4 wk.

Fig. 8 *A* shows daily averages of systolic blood pressure in the group 4 rats before and after transplantation and administration of DOCA-salt. Antihypertensive therapy effectively normalized blood pressure before DOCA-salt treatment. The overall average systolic blood pressure for all six recipients was 122.4±4.6 mmHg during antihypertensive therapy. During the 2-wk period after antihypertensive therapy was discontinued, overall average systolic blood pressure increased to 142.8±4.8 mmHg in all six recipients ($P < 0.05$). In the four recipients then given DOCA-salt, overall average systolic blood pressure increased further to 186.9±8.5 mmHg ($P < 0.001$).

Clearance studies were performed after 25 d of DOCA-salt, and the results were almost identical to those of group 3 rats not pretreated with antihypertensive agents. The GFRs of the transplanted BN kidneys were significantly lower than those of SHR-RT1.N kidneys (BN versus SHR-RT1.N, 0.94±0.6 versus 2.75±1.3 ml/min per kg body wt, $P < 0.02$) (Fig. 8 *B*). Urinary excretion of protein was significantly greater in the BN kidneys than in the SHR-RT1.N kidneys (BN versus SHR-RT1.N, 10.3±4.4 versus 0.6±0.1 μg/min per ml GFR, $P < 0.05$). Moreover, as can be seen in Fig. 8 *C*, the histologic results were almost identical to those of group 3, in that all histologic damage scores were 5–15-fold greater in BN kidneys than in SHR-RT1.N kidneys.

In the two recipients not given DOCA-salt, there was no histologic evidence of vascular damage in either the transplanted BN kidney or the contralateral native SHR-RT1.N kidney, despite 6 wk of exposure to moderate hypertension in the recipients (163–164 mmHg). Glomerular damage, however, was more severe in the BN kidney than in the SHR-RT1.N kidney: 16% versus < 1% for the total glomerular damage scores. Thus, prolonged exposure to even moderate hypertension can produce differential glomerular damage in BN versus SHR kidneys, but exposure to severe DOCA-salt–induced hypertension is required to produce, in 25 d, the characteristic vascular lesions of malignant nephrosclerosis. Finally, at ~ 2.5 mo after transplant, there was no histologic evidence of graft rejection in either of these recipients.

Discussion

To investigate the role of genetic factors in the pathogenesis of hypertension-induced renal damage, we have studied a novel experimental model of SHR in which two genetically different yet histocompatible kidneys are chronically and simultaneously exposed to the same blood pressure profile and

Figure 5. Vascular pathology in BN kidneys. (*A*) Fibrinoid necrosis involving interlobular artery and an afferent arteriole. PAS ×300 (*B*) Vascular myointimal hyperplasia with an onionskin, concentric laminated thickening of the wall of a small artery with severe narrowing of the lumen. Adjacent glomerulus shows ischemic injury. PAS, ×300.

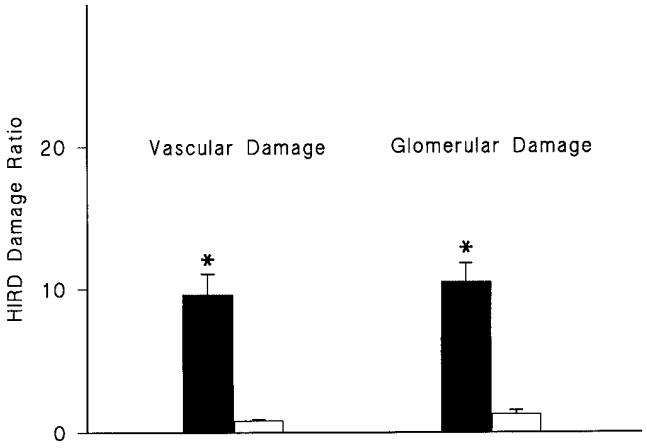


Figure 7. The ratios of hypertension-induced vascular and glomerular damage in the left transplanted kidney/right native kidney are shown for groups 2 and 3: unilaterally nephrectomized SHR recipients of SHR donor kidneys (SHR/SHR) and unilaterally nephrectomized SHR-RT1.N recipients of BN donor kidneys (BN/SHR-RT1.N). See text for explanation of damage scoring. $*P < 0.0001$ maximum versus SHR/SHR ratios. *Black bars*, BN/SHR-RT1.N (n = 8); *white bars*, SHR/SHR (n = 8).

metabolic environment within the same host. In this model, we have found that kidneys from BN rats are much more susceptible to hypertension-induced damage than are those from SHR. The greater damage in BN kidneys versus SHR kidneys could not be attributed to strain differences in transplantation-induced injury, immunologic rejection phenomena, or preexisting strain differences in blood pressure. These findings provide direct support for the hypothesis that genetic factors can determine susceptibility to hypertension-induced renal damage.

In contrast to studies in which susceptibility to hypertension-induced renal damage has been assessed in genetically different individuals that have been assumed to have identical 24-h blood pressure profiles, the current model guarantees that the genetically different kidneys are exposed to the same pressure load over a prolonged period of time. Thus, the model enables us to test for differences in genetic susceptibility to hypertension-induced renal damage despite the large variations in blood pressure that typically occur over both short and long periods of time (12–14). To illustrate the importance of this feature of the model, it is instructive to note the extent to which blood pressure fluctuates in an SHR. Fig. 9 shows the continuous systolic blood pressure tracing for one of the SHR-RT1.N recipients of a BN donor kidney before and during administration of DOCA-salt. It can be seen that systolic blood pressure undergoes diurnal variation, and that it fluctuates rapidly; fluctuations of 100 mmHg are not uncommon. We have observed similar blood pressure fluctuations in SHR that have not undergone renal transplantation. These observations underscore the limitations of conventional blood pressure measurements for investigating the genetic determinants of hypertension-induced target organ damage. Clearly, differences in susceptibility to hypertension-induced organ damage cannot be attributed to genetic factors unless one can strictly control for the enormous variations in blood pressure that occur both within and between individuals (12–14).

The validity of the current model is critically dependent on

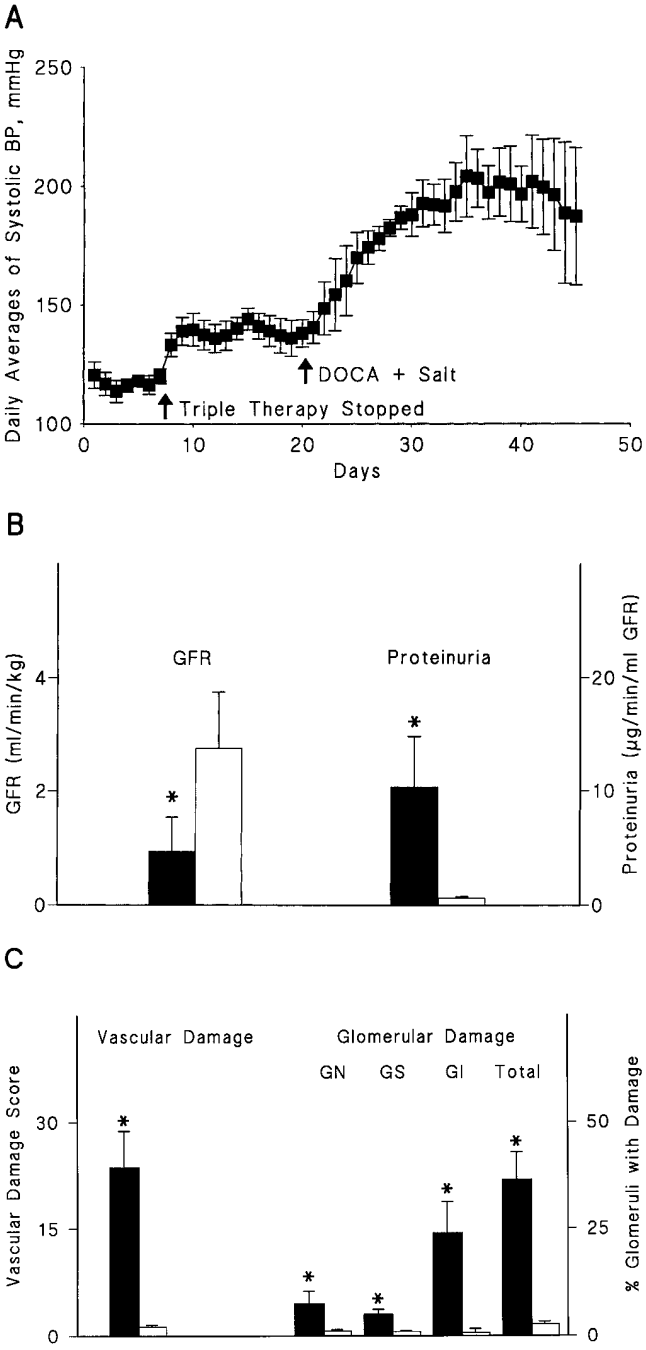


Figure 8. Blood pressure, renal function, and renal histology in unilaterally nephrectomized SHR-RT1.N recipients of BN donor kidneys (group 4; n = 4 males). The SHR-RT1.N recipients were pretreated with antihypertensive therapy to render them normotensive before transplantation. See legend to Fig. 1 for panel descriptions and abbreviations. $*P < 0.05$ maximum, BN kidney versus SHR-RT1.N kidney. *Black bars*, BN kidney; *white bars*, SHR-RT1.N kidney.

the ability to transplant rat kidneys without impairing renal function. Transplantation of BN donor kidneys into BN recipients, or SHR donor kidneys into SHR recipients, did not affect GFR, urinary excretion of protein, or renal morphology. Furthermore, we have shown previously that our transplantation technique does not impair or alter renal function or renal mor-

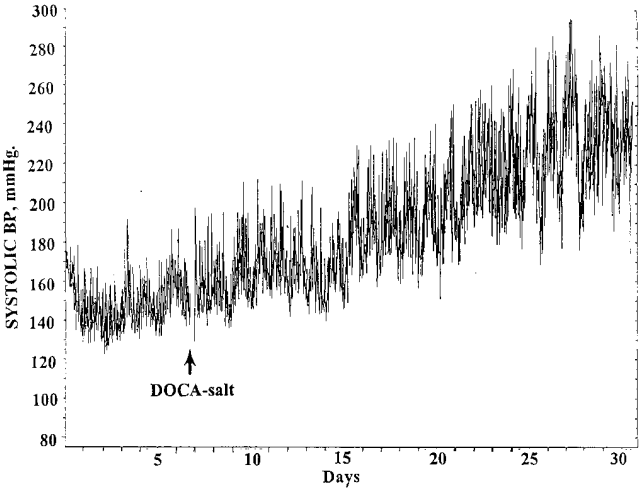


Figure 9. Systolic blood pressure in a unilaterally nephrectomized SHR-RT1.N recipient of a BN donor kidney, before and during administration of DOCA-salt. Each point on this tracing represents the average systolic blood pressure during a 5-s interval, sampled every 10 min, 24 h a day. Note the extreme lability of systolic blood pressure.

phology in normotensive or hypertensive strains of rats (18, 25–28). Specifically, the renal plasma flows and the GFRs of kidneys transplanted into unilaterally nephrectomized recipients are indistinguishable from those of the contralateral native kidneys in the same animals. Thus, it is unlikely that strain differences in transplantation-induced renal damage could account for the differential susceptibility of SHR and BN kidneys to hypertension-induced injury.

In the current cross-transplant studies, we avoided the problem of immunologic rejection by using only donors and recipients that harbor the same MHC. Microscopic analysis of BN kidneys transplanted into SHR-RT1.N recipients showed no evidence of immunologic rejection or damage. Despite the undoubted presence of minor histocompatibility antigens on the BN donor kidney, there was no tubulointerstitial pathology suggestive of rejection at 7–8 wk after transplantation in any of the rats. In many of these rats, severe hypertensive damage could have obscured subtle histologic evidence of rejection. In the BN kidneys transplanted into histocompatible SHR-RT1.N that were not given DOCA-salt, however, hypertensive damage was very mild and confined to glomeruli, and there was no histologic evidence of rejection at 2.5 mo after transplantation. Thus, at least within the time frame of these experiments, the SHR-RT1.N recipients did not mount an immunologic response to minor histocompatibility antigens on the BN kidney vigorous enough to confound interpretation of the results.

To investigate whether the relative resistance of SHR-RT1.N kidneys to hypertension-induced damage was dependent on prior exposure and adapation of the kidneys to high blood pressure, we prevented the development of hypertension in SHR-RT1.N rats before transplantation. Strain differences in susceptibility to hypertension-induced renal damage were not altered by rendering the SHR-RT1.N rats normotensive before study. The results in congenic SHR pretreated with antihypertensive agents (group 4) were nearly identical to the results in congenic SHR not pretreated with antihypertensive agents (group 3). Except for the period of time from birth to weaning, the blood pressure exposures of both BN and SHR-RT1.N kidneys in group 4 were similar before transplantation and identical afterwards. The BN kidneys were exposed to normotensive blood pressure levels from birth to 3–4 wk after transplantation. The SHR-RT1.N kidneys were also maintained in a normotensive environment for the same period of time except for the 20–25-d interval from birth to weaning, when blood pressure is only slightly elevated in the SHR (29). Both BN and SHR-RT1.N kidneys were also exposed to an identical level of moderate hypertension for 2 wk when antihypertensive therapy was discontinued, and both BN and SHR-RT1.N kidneys were exposed to an identical level of severe hypertension during DOCA-salt treatment. Therefore, unless protective adaptation of the SHR-RT1.N kidneys to hypertension occurs before weaning and cannot be reversed by a 10–11-wk exposure to normotensive levels of blood pressure, these observations exclude the possibility that the strain differences in susceptibility to hypertension-induced renal damage are simply secondary to the strain differences in blood pressure prior to transplantation.

We conclude that genetic factors underlie the marked difference in susceptibility to hypertension-induced renal damage between the SHR and BN strains; the BN strain is genetically susceptible, whereas the SHR strain is genetically resistant to hypertension-induced renal injury. The genes that determine this differential susceptibility to hypertension-induced renal damage are unknown. Recent genetic studies in two other strains of hypertensive rats, however, suggest that QTLs influencing the risk for target organ damage may exist on rat chromosome 1. In a linkage study in the Fawn Hooded hypertensive rat, Brown et al. (30) identified two regions of chromosome 1 that contain genes influencing susceptibility to renal failure. At least one of the chromosome regions identified by Brown et al. (30) appears to overlap with a segment of chromosome 1 that has recently been reported to contain a gene influencing susceptibility to stroke in the stroke-prone SHR (31). Taken together, these observations raise the possibility that rat chromosome 1 contains QTLs that increase the risk for hypertension-induced vascular injury in a variety of organs, including the brain and the kidney.

By studying a novel animal model with two kidneys that are genetically different except in the vicinity of the MHC on rat chromosome 20, we have established that the BN rat is inherently more susceptible to hypertension-induced renal damage than is the SHR. In addition to providing direct evidence of a role for genetic factors in the pathogenesis of hypertension-induced renal injury, the current studies establish the feasibility of using organ-specific genome transplants to map QTLs influencing susceptibility to hypertension-induced target organ damage. By performing renal cross-transplants between congenic strains of SHR that differ in single chromosome regions, it should be possible to selectively transfer individual chromosome segments from the BN rat into the kidney of the SHR. This strategy will allow for the creation of SHR with two kidneys that are genetically identical except for defined chromosome segments, and will enable us to investigate directly the role of specific chromosome regions in the pathogenesis of hypertension-induced renal damage in the rat.

Finally, in addition to demonstrating genetic differences between the SHR and BN strains with respect to susceptibility

to hypertensive renal damage, our results also demonstrate genetic differences between these strains with respect to the blood pressure response to DOCA-salt treatment. The SHR is responsive, whereas the BN rat is not. The BN rat, however, is not unique in being unresponsive. Systolic blood pressures of three other rat strains are relatively low after several weeks of DOCA-salt treatment: Wistar Furth, 123±5 mmHg at 5 wk and only 136±2 mmHg at 10 wk (32); Dahl salt resistant, 131±6 mmHg at 4 wk (33); and Long Evans, 133±3 mmHg at 4 wk (34). Interestingly, of these strains that are resistant to DOCA-salt, the Dahl salt resistant rat also is resistant to two-kidney one-clip Goldblatt hypertension (33), whereas the Wistar Furth is susceptible (32). All of these strain differences emphasize the importance of genetics in hypertension.

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References

1. Klahr, S.G., G. Schreiner, and I. Ichikawa. 1988. The progression of renal disease. *N. Engl. J. Med.* 318:1657–1666.

2. Brazy, P.C., W.W. Stead, and J.F. Fitzwilliam. 1989. Progression to renal insufficiency: role of blood pressure. *Kidney Int.* 35:670–674.

3. Walker, W.G. 1993. Hypertension-related renal injury: a major contributor to end-stage renal disease. *Am. J. Kidney Dis.* 22:164–173.

4. Freedman, B.I., S.S. Iskandar, and R.G. Appel. 1995. The link between hypertension and nephrosclerosis. *Am. J. Kidney Dis.* 25:207–221.

5. Rostand, S.G., G. Brown, K.A. Kirk, A.E. Rutsky, and H.P. Dustan. 1982. Racial differences in the incidence and treatment for end-stage renal disease. *N. Engl. J. Med.* 306:1276–1279.

6. Tierney, W.M., C.J. McDonald, and F.C. Luft. 1989. Renal disease in hypertensive adults: effect of race and type II diabetes mellitus. *Am. J. Kidney Dis.* 13:485–493.

7. Cusi, D., G. Tripodi, G. Casari, C. Robba, P. Bollini, G. Merati, and G. Bianchi. 1993. Genetics of renal damage in primary hypertension. *Am. J. Kidney Dis.* 21(Suppl. 2):2–9.

8. Weening, J.J., J.J.B. Beukers, J.D. Grond, and J.D. Elema. 1986. Genetic factors in focal segmental glomerulosclerosis. *Kidney Int.* 29:789–798.

9. Bramdis, A., G. Bianchi, E. Reale, U. Helmchen, and K. Buhn. 1986. Age-dependent glomerulosclerosis and proteinuria occurring in rats of the Milan normotensive strain and not in rats of the Milan hypertensive strain. *Lab. Invest.* 55:234–243.

10. Sterzel, R.B., F.C. Luft, Y. Gao, J. Schnermann, J.P. Briggs, D. Ganten, R. Waldherr, E. Schnabel, and W. Kriz. 1988. Renal disease and the development of hypertension in salt-sensitive Dahl rats. *Kidney Int.* 33:I119–I129.

11. Hampton, J.A., D.A. Bernardo, N.A. Khan, D.A. Lacher, J.P. Rapp, A.F. Gohara, and P.J. Goldblatt. 1989. Morphometric evaluation of the renal arterial system of Dahl salt-sensitive and salt-resistant rats on a high salt diet. II. Interlobular arteries and intralobular arterioles. *Lab. Invest.* 60:839–846.

12. Parati, G., S. Omboni, M. DiRienzo, A. Frattola, F. Albini, and G. Mancia. 1992. Twenty-four hour blood pressure variability: clinical implications. *Kidney Int.* 41:24s–28s.

13. Bidani, A.K., K.A. Griffin, M. Picken, and D.M. Lansky. 1993. Continuous telemetric BP monitoring and glomerular injury in the rat remnant kidney model. *Am. J. Physiol.* 265:F391–F398.

14. Holstein-Rathlou, N.H., J. He, A.J. Wagner, and D.J. Marsh. 1995. Patterns of blood pressure variability in normotensive and hypertensive rats. *Am. J. Physiol.* 269:R1230–R1239.

15. Pravenec, M., P. Klir, V. Kren, J. Zicha, and J. Kunes. 1989. An analysis of spontaneous hypertension in spontaneously hypertensive rats by means of new recombinant inbred strains. *J. Hypertens.* 7:217–222.

16. Sesoko, S., B.L. Pegram, G.W. Willis, and E.D. Frohlich. 1984. DOCA-salt induced malignant hypertension in spontaneously hypertensive rats. *J. Hypertens.* 2:49–54.

17. Bidani, A.K., K.A. Griffin, W. Plott, and M.M. Schwartz. 1994. Renal ablation acutely transforms 'benign' hypertension to 'malignant' nephrosclerosis in hypertensive rats. *Hypertension (Dallas).* 24:309–316.

18. Churchill, M., R. Kline, M. Schwartz, A. Bidani, and P. Churchill. 1990. Kidney transplants in cyclosporine-treated Sprague Dawley rats. *Transplantation (Baltimore).* 49:8–13.

19. Wallenstein, S.C., C.L. Zucker, and J.L. Fleiss. 1980. Some statistical methods useful in Circulation Research. *Circ. Res.* 47:1–9.

20. Ely, D.L., and M. E. Turner. 1990. Hypertension in the spontaneously hypertensive rat is linked to the Y chromosome. *Hypertension (Dallas).* 16:277–281.

21. Grollman, A., T.R. Harrison, and J.R. Williams, Jr. 1940. The effect of various sterol derivatives on the blood pressure of the rat. *J. Pharmacol. Exp. Ther.* 69:149–155.

22. Ouchi, Y., L. Share, J.T. Crofton, K. Iitake, and D.P. Brooks. 1987. Sex difference in the development of deoxycorticosterone-salt hypertension in the rat. *Hypertension (Dallas).* 9:172–177.

23. Selye, H., C.E. Hall, and E.M. Rowley. 1943. Malignant hypertension produced by treatment with desoxycorticosterone acetate and sodium chloride. *Can. Med. Assoc. J.* 49:88–92.

24. Selye, H., and E.I. Pentz. 1943. Pathogenetical correlations between periarteritis nodose, renal hypertension and rheumatic lesions. *Can. Med. Assoc. J.* 49:264–272.

25. Churchill, P., M. Churchill, A. Bidani, and J. Dunbar, Jr. 1993. Streptozotocin-induced renal hemodynamic changes in isogeneic Lewis rats: a kidney transplant study. *Am. J. Physiol.* 264:F100–F105.

26. Churchill, M., P.C. Churchill, M. Schwartz, A. Bidani, and F. McDonald. 1991. Reversible compensatory hypertrophy in transplanted Brown Norway rat kidneys. *Kidney Int.* 40:13–20.

27. Schwartz, M.M., M. Churchill, A. Bidani, and P.C. Churchill. 1993. Reversible compensatory hypertrophy in rat kidneys: morphometric characterization. *Kidney Int.* 43:610–614.

28. Churchill, P.C., M.C. Churchill, A.K. Bidani, and S.F. Rabito. 1995. Kallikrein excretion in Dahl salt sensitive and salt resistant rats with native and transplanted kidneys. *Am. J. Physiol.* 269:F710–F717.

29. Yamori, Y., and J.D. Swales. 1994. The spontaneously hypertensive rat. *In* Textbook of Hypertension. J.D. Swales, editor. Blackwell Scientific Publications, New York. 447–455.

30. Brown, D.M., A.P. Provoost, M.J. Daly, E.S. Lander, and H.J. Jacob. 1996. Renal disease susceptibility and hypertension are under independent genetic control in the fawn-hooded rat. *Nat. Genet.* 12:44–51.

31. Rubattu, S., M. Volpe, R. Kreutz, U. Ganten, D. Ganten, and K. Lindpaintner. 1996. Chromosomal mapping of quantitative trait loci contributing to stroke in a rat model of complex human disease. *Nat. Genet.* 13:429–434.

32. Sciotti, V., and S. Gallant. 1987. Resistance to mineralocorticoid-induced hypertensive vascular disease. *Hypertension (Dallas).* 10:176–180.

33. Dahl, L.K., M. Heine, and L. Tassinari. 1963. Effects of chronic excess salt ingestion. Role of genetic factors in both DOCA-salt and renal hypertension. *J. Exp. Med.* 118:605–617.

34. Hall, C.E., S. Ayachi, and O. Hall. 1973. Hypertension following adrenal enucleation and its absence during desoxycorticosterone treatment in Long-Evans rats. *Endocrinology.* 92:1175–1181.