Hypertension in Rats Induced by Renal Grafts From Renovascular Hypertensive Donors

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Renal transplantations were performed, using microsurgical techniques, with adult male two-kidney, one clip hypertensive rats (n=9) and sham-operated normotensive Wistar-Kyoto rats (n=8) as kidney donors and with F1 hybrids, bred from Wistar-Kyoto and stroke-prone spontaneously hypertensive rat parents, as recipients. Systolic blood pressure before surgery was 200±2.7 mm Hg in hypertensive and 115±1.7 mm Hg in normotensive donors and 144±7.1 and 138±3.5 mm Hg in the two groups of recipients. Renal hypertension in donors was maintained for 14 weeks before surgery was performed and the nonischemic kidneys were transplanted. Bilaterally nephrectomized recipients of renal grafts from hypertensive donors developed sustained hypertension (185±3.9 mm Hg). In contrast, in recipients of renal grafts from normotensive donors, blood pressure decreased significantly to the level of the donors (111±3.7 mm Hg). Posttransplantation hypertension in recipients of renal grafts from hypertensive donors was associated with intrarenal vascular hypertrophy, smaller kidneys, a decreased glomerular filtration rate, an increased plasma urea concentration, and polydipsia as compared with normotensive transplanted controls. Renal pyelograms revealed no gross anatomic alterations of transplanted kidneys. Our data indicate that secondary damage to the renal grafts caused by high perfusion pressure before transplantation can induce hypertension in recipients of these kidneys. Furthermore, our data suggest that renal mechanisms may be necessary to maintain borderline hypertension in F1 hybrids. (Hypertension 1990; 15:429-435)

The underlying mechanisms of primary hypertension are unknown. It is widely believed that an interaction between renal and genetic factors may be important. This hypothesis is supported by several reports demonstrating that blood pressure "travels with the kidney" in renal cross-transplantation experiments in different rat models of genetic hypertension.1-6 The kidney may be involved as a primary or secondary factor in the pathogenic events associated with primary hypertension. In the former case, the kidney is believed to carry a genetic defect, which gives rise to hypertension in predisposed individuals.7-9 In the latter case, the secondary renal sequelae of high perfusion pressure in hypertension of nonrenal origin may become relevant for the perpetuation of the hypertensive disease process or, in case such a kidney becomes a renal graft, for the initiation of hypertension in the recipients. However, it has not yet been directly demonstrated that secondary damage to prospective renal grafts due to high perfusion pressure before transplantation can induce hypertension in recipients of severed kidneys.

We recently reported that adult F1 hybrids, bred from stroke-prone spontaneously hypertensive rat (SHRSP) and Wistar-Kyoto (WKY) rat parents, develop sustained hypertension after bilateral nephrectomy and transplantation of a kidney from an adult SHRSP donor.6 To assess the possible contribution of secondary kidney damage to the development of hypertension in recipients of renal grafts from hypertensive donors, we subjected prospective WKY rat kidney donors to chronic two-kidney, one clip (2K1C) renovascular hypertension. When the nonischemic kidneys of these rats were transplanted to bilaterally nephrectomized adult F1 hybrids, blood pressure in the recipients increased progressively to hypertensive levels, where it chronically remained. In the control group (recipients of renal grafts from...

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sham-operated normotensive donors), blood pressure decreased permanently from borderline hypertensive to normotensive levels.

Methods

Animals

Experiments were conducted with male WKY rats as kidney donors and male F1 hybrids, bred from WKY rat and SHRSP parents, as renal graft recipients. All rats were obtained at the age of weaning from the rat breeding facilities of the University of Heidelberg, Heidelberg, FRG, where SHRSP and WKY rats have been bred since 1975. Animals were housed in plastic cages in a temperature and humidity controlled environment with lights on at 6:00 AM and off at 6:00 PM. Standard rat food (Purina pellets) and tap water were available to the rats ad libitum. All experiments were preapproved by a governmental committee on animal welfare.

Surgery

Renal artery clipping. Renal artery clipping was conducted at the age of 6 weeks. Rats were anesthetized with ether, and a small solid silver clip with an internal diameter of 0.2 mm was placed around the right renal artery through a lateral flank incision. Renal transplantation. The microsurgical technique used in this study has been described in detail elsewhere.6 It is a modification of the technique first described by Fisher and Lee.12 Briefly, a kidney donor and a recipient were anesthetized simultaneously with pentobarbital (60 mg/kg i.p.). The abdomen of the kidney donor was opened by a long midline incision. Renal vessels and ureter of the left kidney were exposed, renal blood circulation was interrupted, and the kidney was immediately flushed with an ice-cold isotonic solution. The kidney was removed, kept cool at all times and transferred to the recipient rat. Kidney recipients were prepared by left unilateral nephrectomy and exposure of the abdominal aorta and vena cava just caudal to the renal vessels through a long abdominal midline incision. Blood flow through the anastomotic area was transected to normotensive levels. The ureter catheter consisted of a single piece of silicone tubing (LHD, Heidelberg, FRG) with an internal and external diameter of 0.3 mm and 0.5 mm, respectively. The procedure for catheter implantation has been described in detail elsewhere.13 Briefly, the graft ureter was exposed through an abdominal midline incision, and the catheter was inserted about 2 mm into the ureter. The free end of the catheter was carefully guided through the lateral abdominal wall and tunneled under the skin to exit at the nape of the neck. The catheter was anchored to the skin of the neck in a way that urine, dripping from its orifice, would fall to the cage floor without staining the rat. After all catheters had been implanted, rats were allowed to recover for 24 hours before measurements of inulin clearance were commenced.

Glomerular Filtration Rate

Glomerular filtration rate (GFR) was determined by measurement of inulin clearance in conscious rats. Rats were instrumented with indwelling vascular and ureteral catheters as described above. Catheters were provided with extension lines, so that they could be handled from outside the cage without disturbing the rat. Rats received an intravenous bolus injection of 120 mg/kg inulin, immediately followed by a continuous intravenous infusion of 2 mg/kg/min inulin. Infusion rate was 17 μl/min for 135 minutes, delivered by an automatic infusion pump (Braun-Melsungen, Melsungen, FRG). Urine was quantitatively collected in preweighed tubes in five consecutive intervals of 15 minutes each, starting after 1 hour of infusion. Urine flow (μl/min) was determined gravimetrically. About 300 μl blood was obtained from the arterial line at the end of each urine collection period. The blood was immediately centrifuged, and the plasma and urine samples were stored at −20°C until assayed.

Inulin concentration was determined photometrically. Fifty microliters plasma and 25 μl urine, respectively, were added to 950 μl (or 975 μl) distilled water plus 200 μl trichlor acetic acid, vortexed, and centrifuged. Five hundred microliters (plasma) or 20 μl (urine) plus 480 μl distilled water of the supernatant were added to 0.5 ml distilled water, 1.0 ml resorcin (at a concentration of 1 mg/ml), and 1.5 ml concentrated HCl. After an incubation period of 25 minutes at 80°C, the optical extinction of the resulting red color complex was measured at 490 nm with a spectrophotometer (Perkin-Elmer, Offenbach, FRG) and compared with a standard curve. All measurements were done in duplicate. The inulin clearance was determined according to standard calculation procedures.
**Plasma Urea Concentration**

Plasma urea concentration was determined photometrically with a commercially available test kit (Ingotest, Boehringer Ingelheim, Ingelheim, FRG). Briefly, urease (10 units/200 μl dissolved in 75% glycerol solution) was added to 20 μl plasma. The resulting ammonia reacts with phenol and hypochlorite in the presence of sodium nitroprusside to form a blue color complex. Optical extinction was measured at 550 nm with a spectrophotometer (Perkin-Elmer) and compared with a standard curve.

**Histology**

Transplanted kidneys were fixed by antegrade vascular perfusion with 4% paraformaldehyde in phosphate-buffered saline at pH 7.4. The kidneys were embedded in paraffin (Paraplast, Monoject Scientific Inc., Athy, Ireland). Sections (3–4 μm) were stained with periodic acid-Schiff and Masson trichrome stain.

**Renal Pyelogram**

Renal pyelograms were performed in rats chronically instrumented with indwelling catheters in femoral artery and vein. Rats were anesthetized with methohexital sodium (Brevimytal, Eli Lilly, Bad Homburg, FRG) intravenously (dosage as needed) and placed under an x-ray apparatus in a supine position. One milliliter contrast medium (Omnipaque, Schering, Berlin, FRG) was injected through the arterial line. x-Rays were taken 5 minutes after the injection of contrast medium.

**Experimental Protocol**

Seventeen young male WKY rats were obtained from the breeding colony and randomly divided in two groups. At the age of 6 weeks, group 1 (n=9) was subjected to 2K1C renovascular hypertension, and group 2 (n=8) was subjected to sham operation. Systolic blood pressure was measured in all rats at weekly intervals by tail plethysmography under light ether anesthesia. At the age of 22 weeks, the left kidney (nonischemic) of each of these rats was transplanted into a randomly selected adult male 22-week-old F1 hybrid rat. During renal transplantation, graft recipients were unilaterally nephrectomized. The second autochthonous kidney was removed 5–7 days later. Blood pressure in graft recipients was also measured at weekly intervals by tail plethysmography. Body weight and water intake were monitored daily in all renal graft recipients throughout the experiment. Before renal transplantation as well as in weeks 3, 5, 7, and 9 after transplantation, 0.5 ml blood was withdrawn from the retro-orbital space of each rat, while it was under light ether anesthesia, for measurements of plasma urea concentration. Ten weeks after transplantation, rats were instrumented with chronic catheters as described above and subjected to either measurements of glomerular filtration rate or renal pyelograms. At the end of the experiments, rats were killed by an overdose of intravenously administered pentobarbital, and the kidneys were removed, decapsulated, and weighed. In addition to the transplanted rats, systolic blood pressure and GFR were also measured in six 32-week-old male F1 hybrids that had been unilaterally nephrectomized at age 22 weeks.

**Statistics**

Results are expressed as mean±SEM. Data were subjected to analysis of variance and post hoc tests where appropriate. Statistical significance was accepted at p<0.05.

**Results**

After unilateral renal artery stenosis, systolic blood pressure rose rapidly in young WKY rats and reached a hypertensive plateau within 2 weeks (Figure 1). Hypertension was maintained from the eighth to the 22nd week of age, at which point the left kidney of the rats was transplanted to F1 hybrid rats. Blood pressure did not change significantly in sham-operated kidney donors. Immediately before renal transplantation, systolic blood pressure was significantly higher in prospective graft recipients (144±7.1 and 138±3.5 mm Hg, respectively, for the two groups) as compared with sham-operated kidney donors (115±1.7 mm Hg). Blood pressure was highest in hypertensive kidney donors (200±2.7 mm Hg). During the first 2 weeks after renal transplantation, blood pressure decreased in both groups (Figure 2). In recipients of kidneys from normotensive donors, this hypotensive development continued until the third week after transplantation. Thereafter, blood pressure stabilized at a low level, which was similar to that of normotensive kidney donors.

In recipients of kidneys from hypertensive donors, blood pressure stopped decreasing and started rising between the second and third week after renal transplantation (Figure 2). This chronic pressor response continued to develop for several weeks until, starting at the sixth week after renal transplantation, blood pressure was significantly elevated over preoperative values. Ten weeks after renal transplantation, systolic blood pressure was 185±4.9 mm Hg in recipients of renal grafts from hypertensive donors, 111±3.7

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**FIGURE 1.** Line graph showing systolic blood pressure in male Wistar-Kyoto rats subjected to either two-kidney, one clip renovascular hypertension or sham operation. Arrow indicates time of surgery.
FIGURE 2. Line graph showing systolic blood pressure in adult male F1 hybrids, bred from Wistar-Kyoto rat and stroke-prone spontaneously hypertensive rat parents. Arrow indicates unilateral nephrectomy and transplantation of a renal graft from either two-kidney, one clip hypertensive or normotensive donors. Second autochthonous kidney was removed 5–7 days later. *p<0.01 compared with presurgical control values.

mm Hg in recipients of renal grafts from normotensive donors and 149±2.4 mm Hg in nontransplanted F1 hybrid controls with solitary kidneys.

Body weight was not significantly different between the two groups at any time throughout the experiment (Figure 3). Daily water consumption increased in both groups after renal transplantation (Figure 4). In recipients of kidneys from normotensive donors, daily water intake reached its maximum during the second week and decreased rapidly during the following 2 weeks to preoperative control levels. In recipients of kidneys from hypertensive donors, daily water intake continued to rise until the third week after surgery and remained elevated throughout the experiment.

Plasma urea concentration increased significantly in both groups after renal transplantation (Figure 5). In recipients of kidneys from normotensive donors, this increase was small and transient and returned to near normal levels during the experiment. In recipients of kidneys from hypertensive donors, plasma urea concentration continued to rise until the fifth week after surgery and remained elevated throughout the experiment.

GFR was significantly reduced in both groups of transplanted rats when compared with unilaterally nephrectomized F1 hybrids with an intact solitary kidney (Figure 6). Renal grafts from normotensive donors had a significantly higher GFR than those from hypertensive donors.

Ten weeks after transplantation, renal grafts from hypertensive donors showed marked-to-severe thickening of the media of small- and medium-sized intrarenal arteries with four to six smooth muscle cell layers and occasional hyalinosis of afferent arterioles. In one kidney, fibrinoid necrosis of an interlobular artery was noted. No such changes were observed in renal grafts from normotensive donors, which showed completely normal intrarenal arterial vasculature.

Renal pyelograms revealed the rather caudal position of transplanted kidneys with an otherwise normal urinary tract. There were no differences between renal pyelograms from normotensive and hypertensive rats except that kidney grafts from hypertensive rats appeared to be smaller. Actual kidney weight at the end of the experiments was 2.02±0.17 g for

FIGURE 3. Line graph showing body weight in bilaterally nephrectomized male F1 hybrids after transplantation of a kidney from either normotensive or two-kidney, one clip hypertensive donors. *p<0.05 compared with presurgical control values.

FIGURE 4. Line graph showing daily water intake in bilaterally nephrectomized male F1 hybrids after transplantation of a kidney from either normotensive or two-kidney, one clip hypertensive donors. *p<0.05 compared with presurgical control values.

FIGURE 5. Line graph showing plasma urea concentration in bilaterally nephrectomized male F1 hybrids after transplantation of a kidney from either normotensive or two-kidney, one clip hypertensive donors. *p<0.05, **p<0.01 compared with presurgical control values. #p<0.05 compared with normotensive group.
transplanted kidneys from 2K1C hypertensive donors and 2.61±0.14 g for kidneys from normotensive donors (p<0.01).

**Discussion**

Our data show a diverging development of blood pressures between recipients of renal grafts from normotensive and 2K1C hypertensive donors (Figure 2), with a sustained decrease in the former and chronic hypertension in the latter group. In the chronic phase after renal transplantation, blood pressures in both groups of renal graft recipients were significantly different from each other and from that in unilaterally nephrectomized F1 hybrid controls, suggesting that chronic blood pressure levels in renal graft recipients were markedly influenced by mechanisms related to the donor kidney.

Posttransplantation hypertension in recipients of renal grafts from hypertensive donors was associated with structural hypertrophy of small- and medium-sized intrarenal arteries, smaller kidneys, a significant decrease in GFR, and an increase in plasma urea concentration as compared with recipients of renal grafts from normotensive donors. Furthermore, between the fourth and ninth week after transplantation, rats in the hypertensive group consumed significantly more water than their normotensive counterparts. The latter finding probably reflects an increased volume loss due to polyuria and may be taken as a further indication for decreased renal function in kidney graft recipients from hypertensive donors. However, these mechanisms are unlikely to have occurred as all renal grafts were taken from the same inbred WKY rat donor strain and transplanted to the same strain of F1 hybrids.

Secondary renal damage due to high perfusion pressure before transplantation has been previously discussed as a possible cause for posttransplantation hypertension in renal graft recipients. However, to our knowledge, this is the first report providing direct experimental evidence in favor of this hypothesis in an animal model of hypertension. Using deoxycorticosterone acetate (DOCA)–salt hypertensive rats as kidney donors, McMillan et al did not find increased blood pressures in renal graft recipients, although hypertension in kidney donors before transplantation was severe and chronically maintained. The reasons for the discrepancy to our data are currently unknown. Possible explanations include differences in 1) the severity or time course of hypertension in kidney donors, 2) the model of hypertension (DOCA vs. 2K1C), and 3) the rat strains used as kidney donors and recipients.

If a rat kidney can be damaged by sustained high perfusion pressure in a way that it will convey hypertension to a renal graft recipient on transplantation, it would be expected that the same kidney will maintain hypertension in a given individual when the hypertensinogenic stimulus is removed. However, in 2K1C hypertensive rats removal of the clip or the ischemic kidney resulted in rapid and sustained reductions of blood pressure to normal or near normal levels. These findings appear to be at variance with the results reported here. A possible explanation for this discrepancy may be that a genetic predisposition could be required together with an injured kidney to initiate hypertension. In fact, F1 hybrids from WKY and SHR parents have been shown to be particularly prone to the development of hypertension if challenged with stimuli that would not be hypertensinogenic in other rat strains with a purely normotensive genetic background.

The normalization of systolic blood pressure after renal transplantation in recipients of kidneys from normotensive donors suggests a major role for renal mechanisms in maintaining elevated blood pressures in F1 hybrids. A significant contribution of renal mechanisms to the maintenance of genetically induced hypertension has also been suggested by renal transplantation studies in other hypertensive rat strains. Thus, in the Dahl strain, Dahl et al
reported that rats with the genetic propensity to the development of hypertension did not become hypertensive when their kidneys were replaced by a solitary renal graft from the salt-resistant substrain and when they were on a low salt diet. In contrast, unilaterally nephrectomized salt-sensitive Dahl rats and their peers with autologous renal transplants did develop moderate hypertension despite the fact that they were maintained on a low salt diet. However, when the recipients of renal grafts were placed on a high salt diet, all groups developed some degree of hypertension regardless of the genetic background of the different donor–recipient combinations. Similarly, Bianchi et al. reported permanent blood pressure normalization in Milan hypertensive rats on a normal salt diet after bilateral nephrectomy and transplantation of kidneys from a normotensive control strain. When switched to a high salt diet, the same animals developed moderate increases in blood pressure. Together, these findings indicate that genetically induced hypertension in two different animal models is largely maintained by, although not solely dependent on, genetically determined renal mechanisms. A role for a genetic renal defect in primary hypertension is further supported by a study in patients with essential hypertension and subsequent end-stage renal failure whose blood pressures were permanently normalized by bilateral nephrectomy and transplantation of kidneys from normotensive donors. The nature of the putative hypertensinogenic mechanisms in F1 hybrid kidneys is currently unknown. Possible mechanisms include 1) production or decreased inactivation of a hypertensive factor, 2) lack of production of a hypotensive factor, and 3) disturbances of long-term sodium balance and volume homeostasis.

Taken together, our data provide direct evidence in rats that high perfusion pressure can damage prospective renal grafts before transplantation in a way that will give rise to hypertension in genetically predetermined, bilaterally nephrectomized recipients of such kidneys. Furthermore, our data suggest that renal mechanisms may be necessary to maintain borderline hypertension in F1 hybrids bred from SHRSP and WKY rat parents. Whereas the first result illustrates the potential importance of secondary renal mechanisms in the pathogenesis of hypertension, the second finding at the same time points toward the possibility of a primary defect residing within the F1 hybrid kidney.

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**KEY WORDS**

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- posttransplantation hypertension
- Wistar-Kyoto rat
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