Sodium Retention and Hypertension After Kidney Transplantation in Rats

Christiane Graf, Christiane Maser-Gluth, Willem de Muinck Keizer, and Rainer Rettig

The present study was designed to investigate the development of blood pressure and renal sodium handling in recipients of renal grafts from adult stroke-prone spontaneously hypertensive rats (SHRSP), normotensive Wistar-Kyoto (WKY) rats, and borderline hypertensive F$_1$ hybrids bred from SHRSP and WKY rats. Unilaterally nephrectomized F$_1$ hybrids served as renal graft recipients. The second native kidney was removed 7 days after transplantation. Starting on the day of transplantation, renal graft recipients were put on a standard diet for 7 days followed by a low salt diet (0.18% salt) for 10 days and a high salt diet (1.8% salt) for another 14 days. In recipients of a renal graft from SHRSP donors, systolic blood pressure rose progressively from 140±4 mm Hg before to 190±7 mm Hg 4 weeks after transplantation. In contrast, in recipients of a renal graft from WKY rat donors, blood pressure fell during the same time from 139±7 mm Hg to 120±4 mm Hg. Blood pressure did not change significantly in recipients of a renal graft from F$_1$ hybrid donors (132±4 versus 138±7 mm Hg). With transition from a low salt to high salt diet, all rats exhibited renal sodium retention. The accumulating amount of sodium retained by the renal graft was significantly higher in recipients of an SHRSP kidney than in recipients of a WKY rat kidney at all days on the high salt diet. It was also higher in recipients of an SHRSP kidney versus recipients of an F$_1$ hybrid kidney on days 8 through 14 on the high salt diet and in recipients of an F$_1$ hybrid kidney versus recipients of a WKY rat kidney on days 1 through 5 on the high salt diet. On the low salt diet, urinary aldosterone excretion was lower in recipients of an SHRSP kidney compared with recipients of a WKY rat kidney. On the high salt diet, it was similar in both groups. Urinary corticosterone excretion was similar in both groups on the low salt diet and increased significantly on the high salt diet in recipients of an SHRSP kidney but not in recipients of a WKY rat kidney. At the end of the protocol, glomerular filtration rate, renal plasma flow, plasma sodium concentration, and fractional sodium excretion were not significantly different among groups. These results confirm our previous finding that recipients of a renal graft from SHRSP donors developed posttransplantation hypertension, whereas blood pressure actually decreased in recipients of a renal graft from WKY rat donors. In addition, the data demonstrate that posttransplantation hypertension in recipients of an SHRSP kidney is associated with increased renal sodium retention. (Hypertension 1993;21:724–730)

KEY WORDS • hypertension, primary • kidney transplantation • sodium • rats, inbred SHR

The pathogenesis of primary hypertension is not completely understood. There is evidence that the initial trigger for the development of an elevated blood pressure may reside within the kidney (for review, see References 1–4). This evidence is largely derived from renal cross-transplantation studies between genetically hypertensive and normotensive rat strains as well as from retrospective analyses in human renal graft recipients. These studies have unanimously shown that hypertension can be transferred with the kidney from a genetically hypertensive donor to a normotensive recipient.$^{5-16}$ Most$^{2,5,7,11,13}$ but not all$^{12,14}$ studies also showed that bilateral nephrectomy and subsequent transplantation of a kidney from normoten-

From the Department of Pharmacology, University of Heidelberg, and the German Institute for High Blood Pressure Research, Heidelberg (FRG).

Supported by a grant from the Deutsche Forschungsgemeinschaft (Re 5227/1-1).

Address for correspondence: Rainer Rettig, MD, Department of Pharmacology, University of Heidelberg, Im Neuenheimer Feld 366, D-6900 Heidelberg, FRG.

Received August 12, 1992; accepted in revised form January 5, 1993.
Renal sodium handling in bilaterally nephrectomized recipients of a kidney from SHR donors, who develop posttransplantation hypertension, and in recipients of a kidney from WKY donors, who remain normotensive, has not been previously investigated. The present study was designed to test the hypothesis that posttransplantation hypertension in bilaterally nephrectomized recipients of a kidney from stroke-prone SHR (SHRSP) donors is associated with increased renal sodium retention compared with recipients of an (SHRSP × WKY) F1 hybrid (F1H) or a WKY kidney. In addition, we measured glomerular filtration rate and renal plasma flow as well as 24-hour urinary protein, aldosterone, and corticosterone excretion in renal transplanted rats with and without posttransplantation hypertension.

Methods

Animals

Experiments were conducted in adult male SHRSP, normotensive WKY rats, and F1H bred from SHRSP and WKY parents as kidney donors as well as in male F1H as renal graft recipients. Rats were obtained at birth from the rat breeding facilities of the University of Heidelberg, FRG, where inbred WKY and SHRSP strains have been maintained since 1975.22 Animals were housed in plastic cages in a temperature- and humidity-controlled environment with lights on at 6 AM and off at 6 PM. If not otherwise indicated, standard rat food (Altromin pellets) containing 0.6% NaCl and tap water were available to the rats ad libitum. All experiments were preapproved by a governmental committee on animal welfare.

Surgery

Renal transplantation. The microsurgical technique used in this study has been described in detail elsewhere24 and is a modification of the technique first described by Fisher and Lee23 and Lee.24 Briefly, a kidney donor and a recipient were anesthetized simultaneously with pentobarbital (60 mg/kg i.p.). The abdominal incision. Blood flow through the anastomotic area was transversely interrupted, and the grafted blood vessels were anastomosed end-to-side to the abdominal aorta and vena cava of the recipient rat. During surgery, the graft was repeatedly rinsed with ice-cold saline. The recipient was prepared by performing left unilateral nephrectomy and exposing 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 transversely interrupted, and the grafted blood vessels were anastomosed end-to-side to the abdominal aorta and vena cava of the recipient with 9-0 polyamide suture material (Ethilon, Ethicon Co., Norderstedt, FRG). The grafted ureter was directly inserted into the recipient's urinary bladder through a small hole in the bladder wall. Total graft ischaemia lasted 40–50 minutes. After surgery, rats were treated with 50 mg i.p. ampicillin (Binotal, Bayer AG, Leverkusen, FRG) per rat per day for 10 days.

Vascular and ureter catheters. For clearance measurements, rats were instrumented while under ether anesthesia with chronic indwelling catheters in the right femoral artery and vein as well as in the graft ureter. The femoral artery catheter consisted of two pieces of PE-10 and PE-50 tubing (Portex Corp., Hythe, UK) sealed together under hot air. The catheter for the femoral vein was a single piece of PE-25 tubing. Both catheters were inserted at a length of approximately 3 cm into the respective blood vessels and tunneled under the skin to exit through the scruff of the neck. When not in use, catheters were filled with heparinized saline (20 units/mL) and closed with a stainless-steel pin.

The ureter catheter was a single piece of silicone tubing (LHD, Heidelberg, FRG) with internal and external diameters of 0.3 and 0.5 mm, respectively. The procedure for catheter implantation has been described in detail elsewhere.25 Briefly, the graft ureter was exposed through an abdominal midline incision, and the catheter was inserted approximately 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 animal. After all catheters had been implanted, animals were allowed to recover for 48 hours before measurements were begun.

Measurements of Glomerular Filtration Rate and Renal Plasma Flow

Glomerular filtration rate and renal plasma flow were determined as inulin and para-aminomhippuric acid clearances in conscious rats. Animals were instrumented with indwelling vascular and ureteral catheters as described above. Catheters were provided with extension lines to be handled from outside the cage without disturbing the animal. Rats received a 1-mL i.v. bolus injection, containing 40 mg inulin and 5 mg para-aminomhippuric acid, immediately followed by a continuous intravenous infusion of 2 mg/kg per minute inulin and 0.25 mg/kg per minute para-aminomhippuric acid. Infusion volume was 17 μL/min for a total infusion time of 135 minutes, delivered by an automatic infusion pump (Braun-Melsungen, FRG). Urine was quantitatively collected in preweighed tubes in five consecutive sampling intervals of 15 minutes each, starting after 1 hour of infusion. Urine flow (microliter per minute) was determined gravimetrically. At the end of each urine collection period, approximately 300 μL of blood was obtained from the arterial line. Plasma and urine samples were stored at −20°C until assayed. Inulin and para-aminomhippuric acid concentrations in plasma and urine were determined photometrically. All measurements were done in duplicate. Inulin and para-aminomhippuric acid clearances were determined according to standard calculation procedures.

Blood Pressure

Before renal transplantation, blood pressure was measured by tail plethysmography with donor and recipient rats under light ether anesthesia. After transplantation, these measurements were repeated at weekly intervals in renal graft recipients. All readings were taken by the same person, who was blinded with...
respect to the experimental groups. In addition, in renal graft recipients, blood pressure was directly measured at the end of the protocol via an arterial line. The arterial line was connected to a Statham P23Db pressure transducer, a Gould Brush pressure computer, and a Gould Brush 2400 recorder (all Gould Inc., Oxnard, Calif.). During these measurements, rats were conscious and unrestrained in their home cages.

**Urinary Sodium, Protein, and Steroids**

Sodium concentrations were determined by flame photometry. Urinary protein concentration was determined with the Lowry method. For measurements of steroids, urine samples were extracted with ethyl acetate and the extracts purified by paper chromatography. After purification, aldosterone and corticosterone were quantified with specific radioimmunoassays as previously described.

**Experimental Protocol**

The left kidneys from 12 SHRSP, 14 WKY rats, and 13 F1,H donors were removed and transplanted to 39 unilaterally nephrectomized F1,H. Renal graft recipients were returned to their home cages and placed on a standard rat diet containing 0.60% NaCl. Seven days after renal transplantation, the remaining native kidney was also removed from renal graft recipients, and the animals were transferred to metabolic cages. At the same time, they were put on a low salt diet (0.18% NaCl) for 10 days, immediately followed by a high salt diet (1.80% NaCl) for another 14 days (Figure 1). During special salt diets, rats were offered distilled water instead of tap water as drinking fluid. The diets consisted of sodium-free granular rat chow supplemented with NaCl according to the protocol. The sodium content of the diet was verified by flame photometry. Every day, a known amount of chow was offered to each rat in a special trough equipped with a food trap to minimize spillage. After each 24-hour period, food remaining in the trough or the food trap was weighed and 24-hour food consumption was calculated. While in metabolic cages, rats had access to distilled water ad libitum. Daily sodium intake was calculated as the product of sodium concentration in the chow times the amount of chow consumed. Twenty-four-hour urine samples were quantitatively collected from each rat in volumetric cylinders containing mineral oil. Urine volume was recorded, and samples were stored at −20°C until further analysis. Daily urinary sodium excretion was calculated as the product of urinary sodium concentration times 24-hour urinary volume.

**Statistics**

Data are expressed as mean ± SEM. Statistical comparisons were made as indicated either by Student's *t* test for unpaired samples, by one-way analysis of variance, or by two-way analysis of variance with repeated measures on one factor where "time" was the within-subjects factor and "donor strain" the between-subjects factor. When appropriate, analysis of variance was followed by post hoc Newman-Keuls tests. Statistical significance was accepted at a value of *p*<0.05.

**Results**

Before transplantation, systolic blood pressure (Figure 2) was 212±8 mm Hg in SHRSP donors, 108±3 mm Hg in WKY donors, and 140±4 mm Hg in F1,H donors. Systolic blood pressures in the three groups of F1,H recipients were similar (140±4, 138±7, and 132±4 mm Hg). After transplantation, systolic blood pressure in recipients of an SHRSP kidney rose to 151±10 mm Hg during week 2 when they were on the low salt diet and further to 190±7 mm Hg during week 4 when they were on the high salt diet. In contrast, systolic blood pressure in recipients of a WKY kidney decreased to 107±3 mm Hg during the second week after transplantation. When recipients of a WKY kidney were switched to the high salt diet, systolic blood pressure rose slightly to 120±4 mm Hg but remained significantly below presurgical values. In recipients of an F1,H kidney, blood pressure fell transiently after transplantation and returned to presurgical levels at the third week, where it remained throughout the study. Blood pressure read-
ings obtained by tail plethysmography with rats under light ether anesthesia were confirmed by direct measurements in conscious animals at the end of the protocol (Table 1). In general, directly measured systolic blood pressures tended to be approximately 15 mm Hg higher than the tail-cuff readings. During the low salt diet, all animals achieved an even sodium balance. Abrupt transition to a diet containing 10 times more sodium resulted in a positive sodium balance. At the end of the protocol, fractional sodium excretion rose significantly in recipients of an SHRSP kidney while on the low salt diet, versus recipients of a WKY kidney on days 1 through 14 on the high salt diet.

At the end of the protocol, fractional sodium excretion (Table 1) was somewhat lower in recipients of an SHRSP kidney than in the other two groups, but this difference was not statistically significant. Glomerular filtration rate, renal plasma flow, and plasma sodium concentration were not significantly different among groups (Table 1). Twenty-four-hour urinary protein excretion was significantly greater in recipients of an SHRSP kidney than in the other two groups (Table 1).

On transition from the low to high salt diet, daily food intake (Figure 4) decreased significantly in all rats. During the high salt diet, daily food intake remained relatively stable at a low level, with significantly less food consumption in recipients of an SHRSP kidney than in the other two groups.

Body weight (Figure 5) increased in parallel in all three groups during the low salt diet. Placing the animals on the high salt diet for 14 days resulted in a reduced weight gain in recipients of a WKY kidney and in recipients of a renal graft from F1 donors. At the same time, body weight in recipients of an SHRSP kidney did not change significantly from the value obtained during the low salt diet.

On the low salt diet, 24-hour urinary aldosterone excretion (Figure 6, top panel) was significantly higher in recipients of a WKY kidney compared with recipients of an SHRSP kidney. When fed the high salt diet, both groups had low urinary aldosterone levels, with no significant difference between the groups. Twenty-four-hour urinary corticosterone excretion (Figure 6, bottom panel) was similar in recipients of a WKY kidney and recipients of an SHRSP kidney while on the low salt diet. When fed the high salt diet, urinary corticosterone excretion rose significantly in recipients of an SHRSP kidney but not in recipients of a WKY kidney.
Discussion

In (SHRSP x WKY)-F1H renal transplantation combined with bilateral nephrectomy had differential effects on long-term blood pressure levels, depending on the source of the graft. Recipients of an SHRSP kidney developed posttransplantation hypertension, whereas blood pressure actually decreased in recipients of a WKY kidney. Transplantation of an F1H kidney to an F1H recipient resulted in an acute transient decrease with no significant change in long-term arterial pressure. The differences in long-term posttransplantation blood pressure levels among groups as determined by the tail-cuff method were essentially confirmed by direct blood pressure measurements through indwelling arterial catheters at the end of the experiments. In general, absolute values of systolic blood pressure were somewhat higher when measured directly than with the indirect method. This difference is most likely due to the effects of anesthesia during tail plethysmography.

The finding of posttransplantation hypertension in recipients of an SHRSP kidney is in keeping with several reports from this13-15 and other16 laboratories. Some16,17 but not all13,14 of these studies also found that blood pressure decreased to normotensive levels in borderline hypertensive recipients of a WKY kidney. Here, we report for the first time that long-term blood pressure in F1H was not significantly altered after bilateral nephrectomy and subsequent transplantation of an F1H kidney. This finding is in contrast to a previous study by Kawabe et al,11 who reported that blood pressure in recipients of an (SHR x WKY)-F1H kidney decreased significantly and remained low for several weeks. The reasons for this discrepancy are currently unknown. It should be noticed, however, that in the present study renal graft function was well preserved and tail-cuff readings of systolic blood pressure were confirmed by direct intra-arterial measurements. It is conceivable that sodium retention during the high salt diet may have contributed to the blood pressure normalization after a transient decrease in recipients of an F1H kidney in our study. A recent report that a high salt diet increases blood pressure in (SHR x WKY)-F1H is in keeping with this notion.26

Total body sodium has been proposed to be regulated about different steady states that vary depending on the intake of sodium.26 At the end of the low salt regimen, all rats had reached an even sodium balance. Abrupt transition to a high salt diet resulted in renal sodium retention in all rats, probably reflecting a qualitatively normal regulatory response to adjust total body sodium to a higher level.30 The accumulating amount of sodium retained by the renal graft during 2 weeks on a high salt diet was significantly higher in recipients of an SHRSP kidney than in both of the other groups. Increased renal sodium retention in hypertensive F1H recipients of an SHRSP kidney compared with normotensive F1H recipients of a WKY kidney has not been previously reported. It may be a causal factor contributing to posttransplantation hypertension in recipients of a renal graft from genetically hypertensive donors.31 Alternatively, it may be a consequence of32 or unrelated to33 hypertension. Although our study does not allow us to distinguish between these possibilities, it clearly demonstrates that renal sodium retention was elevated in recipients of an SHRSP kidney compared with recipients of a WKY kidney despite a progressively increasing arterial pressure in the former. In addition, borderline hypertensive recipients of an F1H kidney tended to retain more sodium than normotensive recipients of a WKY kidney (statistically significant on days 1–5 on the high salt diet).

The mechanisms underlying the increased retention of dietary sodium in recipients of an SHRSP kidney are currently unknown. Renal sodium excretion is regulated by a complex interaction of multiple effector systems. Because the different renal grafts were transplanted into the same homeostatic environment of F1H recipients, it is likely that factors intrinsic to the kidney play a major role in this process. These factors may include primary alterations in glomerular hemodynamics, tubular...
lar sodium handling, or both. In the present study, there were no statistically significant differences among groups in glomerular filtration rate and renal plasma flow, confirming previous results from similar studies.\textsuperscript{14,15} If anything, glomerular filtration rate tended to be lower in recipients of a WKY kidney than in rats with a renal graft from SHRSP donors. It is therefore unlikely that a decrease in glomerular filtration rate in recipients of an SHRSP kidney may have contributed to increased renal sodium retention in these rats.

Fractional sodium excretion (percent of filtered sodium across the glomeruli) was somewhat less in recipients of an SHRSP kidney than in recipients of a WKY or F,H kidney when the animals were in sodium balance or close to an even sodium balance at the end of the experiments. However, this difference did not reach statistical significance. Thus, the mechanisms underlying increased sodium retention in recipients of an SHRSP kidney on a high salt diet remain unclear. They may be more favorably studied during maximum sodium retention in the early phase of a high salt diet.

Sodium excretion is also affected by circulating factors such as aldosterone and other hormones. Although it must be assumed that aldosterone levels were similar in all groups of F,H before transplantation, the effects of renal grafting may have influenced this parameter. Because plasma steroid levels are subject to considerable circadian variation, long-term alterations in these parameters are best detected by measuring 24-hour urinary excretion. In the present study, 24-hour urinary aldosterone excretion was significantly lower in recipients of an SHRSP kidney than in recipients of a renal graft from WKY donors on a low salt diet. This may reflect a tendency of renal grafts from SHRSP donors to retain sodium even when dietary sodium content is rather low. The finding that recipients of an SHRSP kidney did not further reduce urinary aldosterone excretion on the high salt diet consistent with this interpretation. In contrast, in recipients of a WKY kidney, the high salt diet led to a significant suppression of aldosterone, probably in response to the long-term sodium load. Two-hour urinary excretion of corticosterone was not significantly different between recipients of a WKY kidney and recipients of an SHRSP kidney on the low salt diet. When placed on the high salt diet, recipients of an SHRSP kidney but not recipients of a WKY kidney responded with a significant increase in 24-hour corticosterone excretion. The reasons for this differential response between the two groups are not directly apparent to us. Corticosterone is the major glucocorticoid in rats and as such may respond to stress.

The finding that corticosterone levels were similar between recipients of an SHRSP kidney and recipients of a WKY kidney while they were on the low salt diet indicates that bilateral nephrectomy and implantation of an SHRSP kidney by itself was no more stressful to the recipients than bilateral nephrectomy followed by implantation of a WKY kidney. On the other hand, the presence of an SHRSP kidney in F,H did have several consequences, such as an increase in blood pressure and a more marked reduction in food intake on the high salt diet, which were different from the effects caused by a transplanted WKY kidney. According to the present results, the long-term effects of an SHRSP kidney in F,H may include an increased corticosterone release in response to a high salt diet. The mechanisms underlying this response remain to be elucidated.

In the present study, body weight increased only slightly during the high salt diet in all groups of transplanted rats despite significant renal sodium retention. There are several explanations for this discrepancy. First, a substantial amount of sodium may have been excreted via the feces. In adult WKY rats and SHR on a standard diet containing 0.5% salt, the fraction of sodium excreted via the feces has been reported to be approximately one third of the total amount excreted, with no significant differences between the two strains.\textsuperscript{19} Furthermore, in SHR, the absolute amount of sodium excreted via the feces appears to be relatively stable over a wide range of dietary salt intake.\textsuperscript{24} Second, daily food intake decreased considerably in all groups when rats were switched from a low to a high salt diet. The decrease in daily food intake was largest in recipients of an SHRSP kidney, which ate approximately 20% less food than rats in the other two groups. Despite less food intake, body weight increased consistently in all rats on the first day of the high salt diet, when renal sodium retention was most prominent. A third explanation for the failure to gain more weight during the high salt diet may be the significant proteinuria that occurred in recipients of an SHRSP kidney. In a recent study in rats,\textsuperscript{35} puromycin aminonucleoside-induced proteinuria of 400-500 mg/day accounted for a deficit in body mass of approximately 50 g. Although in our study proteinuria was far less severe, it may still be partly responsible for the failure of sodium-retaining recipients of an SHRSP kidney to gain more body weight during a high salt diet than recipients of an F,H or WKY kidney.

Taken together, our data show that posttransplantation hypertension in recipients of an SHRSP kidney is associated with increased renal sodium retention on a high salt diet. Although the relation between increased renal sodium retention and posttransplantation hypertension cannot be determined by the present study, it is clear that recipients of an SHRSP kidney suffering from frank hypertension retained more sodium while on a high salt diet than borderline hypertensive recipients of an F,H kidney and normotensive recipients of a WKY kidney. In addition, recipients of an F,H kidney retained more sodium than recipients of a WKY kidney during the first 5 days on a high salt diet. These data are compatible with the hypothesis that increased renal sodium retention contributes to posttransplantation hypertension in recipients of an SHRSP kidney.

Acknowledgment

We thank Manuela Ritzal for expert technical assistance.

References


Sodium retention and hypertension after kidney transplantation in rats.
C Graf, C Maser-Gluth, W de Muinck Keizer and R Rettig

Hypertension. 1993;21:724-730
doi: 10.1161/01.HYP.21.5.724

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/21/5/724