Renal Transplantation Between Male and Female Spontaneously Hypertensive Rats

Stephen B. Harrap, Bao-Zhong Wang, and Donald G. MacLellan

The higher blood pressures of male compared with female spontaneously hypertensive rats (SHR) are the result of the inheritance of different sex chromosomes, although the pathophysiology has not been defined clearly. The reported hypertensive effect of kidneys transplanted from male SHR raises the possibility of a sex-specific renal abnormality, but the effects of transplanting female SHR kidneys have not been studied. To test this hypothesis, single kidneys were transplanted from male SHR into female SHR recipients and vice versa, followed by removal of the native kidneys of the recipients. Male and female SHR that had undergone uninephrectomy were used as controls. After surgery at 14 weeks of age, systolic blood pressures were measured each week until 30 weeks of age. The replacement of a SHR female kidney with a SHR male kidney was not associated with any significant rise in blood pressure, and the replacement of a SHR male kidney with a kidney from a female SHR was not associated with any reduction in blood pressure. These results indicate that the sexual dimorphism of SHR blood pressure is not the result of intrinsic renal differences between males and females and that nonrenal factors would be more likely to explain the blood pressure differences between the sexes. (Hypertension 1992;19:431–434)

Key Words • blood pressure • genetics • kidney • transplantation • Y chromosome • spontaneously hypertensive rats

The blood pressure of male spontaneously hypertensive rats (SHR) is significantly higher than that of female SHR, and genetic studies have revealed important blood pressure effects of the sex chromosomes.1–3 In general, the sexual dimorphism of SHR blood pressure may be due to hypertensive effects of the Y chromosome in males, sex-influenced autosomal loci,1,2 and/or blood pressure–lowering effects of the X chromosomes in females.3 However, the exact pathophysiological mechanisms by which the sex chromosomes exert their effects are unknown. Renal abnormalities are likely genetic mechanisms in SHR hypertension,4 and it is possible that hypertensive effects that have been demonstrated after transplantation of the male SHR kidney5,6 depend on the influence of the SHR Y chromosome. However, the blood pressure effects of transplanting the female SHR kidney have not been studied, and this question remains unresolved.

This project was designed to test the hypothesis that the difference in blood pressure between male and female SHR is due to inherent differences between the kidneys of the two sexes. According to this hypothesis, replacement of the female kidney with that of a male should cause blood pressure to rise, and substitution of the male kidney with that of a female should cause blood pressure to fall.

Methods

SHR used in this study were derived from a colony of SHR in the Experimental Breeding Laboratory of the Genetic Physiology Unit. These animals are direct descendants of SHR from the National Institutes of Health, Bethesda, Md., and inbreeding, which is checked regularly using polymorphic markers, has been maintained by strict brother-sister mating. All procedures used in these studies were approved by the Austin Hospital Animal Welfare Committee.

At 10 weeks of age, 18 female and 14 male SHR entered the study. To accustom the rats to blood pressure measurement, training was undertaken at 11 and 12 weeks of age. Rats were preheated to about 27°–32°C, placed in a clear Perspex restraining cage, and fitted around the tail (model 12-28 BP system, IITC Life Science, Calif.) with a combined pressure cuff/pulse sensor device. Pretransplant systolic blood pressure was measured at 13 weeks of age and then each week after transplantation until 15 weeks posttransplantation. All rats received water and standard laboratory chow, and body weight was measured weekly.

Renal transplantation was performed at 14 weeks of age. This timing was chosen for two reasons. First, although phenotypic renal abnormalities7 are demonstrable in the kidneys of young SHR, transplantation of kidneys from either young or old SHR exert similar hypertensive effects in normotensive donors.5 Second, the recently described blood pressure effects of a gene or genes on the SHR Y chromosome are not apparent until after about 13 weeks of age.6,8

Surgery was performed according to previously published methods5,6,9 with some modifications. Previous studies with this technique (see details below) in our
vascular anastomoses were completed in 15 minutes and the perfusion of the graft after releasing the clamps was controlled by gentle local pressure. In all cases, the kidney was uniformly pale and the renal vein effluent clear. Warm ischemia time was always less than 30 seconds. The renal artery was then cut just distal to the catheter insertion point, and the kidney was removed carefully in the same manner as the donor kidney and ureter, and the renal vessels were mobilized after ligation and cutting of the left adrenal and testicular arteries and veins. The ureter was cut one third of the way down its length since this section receives its blood supply from the renal artery. The distal end of the ureter was cannulated with a short segment of PE-10 tubing to be used as an internal stent for later anastomosis. The renal artery was occluded as close as possible to the aorta with a microvascular clamp, and a small incision was made in the proximal renal artery, through which a PE-10 catheter was introduced to permit in situ perfusion of the kidney with ice-cold 150 mmol/l sodium chloride solution containing heparin (100 units/ml). The left renal vein was cut close to its junction with the vena cava, and perfusion was continued (total 1–2 ml) until the kidney was uniformly pale and the renal vein effluent clear. Warm ischemia time was always less than 30 seconds. The renal artery was then cut just distal to the catheter insertion point, and the kidney was removed and placed in ice-cold saline solution where further dissection of surrounding connective tissue and preparation and trimming of the renal vessels were undertaken. Donor animals were allowed to recover and were used as uninephrectomy controls during the remainder of the experiment.

Recipient rats were also anesthetized with pentobarbital (60 mg/kg i.p.) for insertion of polyethylene catheters (PE-50) into the left carotid artery. These catheters were exteriorized in the interscapular region, and rats were allowed to recover in individual cages overnight with free access to food and water. The following morning, between 9 AM and 11 AM, rats remained in their own cages and a blood pressure transducer (model DPT 3003-S, Peter von Berg, Munich, FRG) was attached to the infrarenal aorta via an additional length of PE-50 tubing. In all rats studied, the catheters demonstrated free back-flow of blood and an obvious pulse wave. Transducer signals were preamplified through a preamplifier (model 7C, Grass Instrument Co., Quincy, Mass.) before analog-digital signal conversion (Maclab/8, Analog Digital Instruments Pty Ltd, Castle Hill, Australia) for the recording, storage, and off-line analysis of data. Up to four animals were studied at one time, and the calibration of each transducer was checked daily. Once the animals were resting quietly and blood pressure appeared stable, recordings were made for half an hour with signal sampling every 0.25 seconds, and the average of these readings was used to estimate mean arterial blood pressure.

Once blood pressure had been measured, arterial blood was drawn for measurement of plasma creatinine and urea, after which the rats were killed by an overdose of pentobarbital, and their hearts and kidneys were removed immediately and weighed fresh.

Statistical Analysis

Longitudinal blood pressure and weight data were compared between groups using repeated-measures analysis of variance (see Reference 10) (MANOVA, SPSS/PC+). Cross-sectional comparisons were made between transplanted and control animals of each sex using Student’s t test for independent groups.

Results

There were no significant overall differences in body weight between control and transplanted rats of either sex.
TABLE 1. Variables Measured at 30 Weeks of Age in Female and Male Control and Transplanted Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Variables</th>
<th>Female Control</th>
<th>Female Transplanted</th>
<th>Male Control</th>
<th>Male Transplanted</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>6</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>228 (220–236)</td>
<td>228 (214–243)</td>
<td>416 (390–442)</td>
<td>419 (392–447)</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>186 (180–191)</td>
<td>177 (164–190)</td>
<td>205 (194–216)</td>
<td>205 (196–214)</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>0.983 (0.939–1.026)</td>
<td>0.995 (0.929–1.061)</td>
<td>1.543 (1.426–1.606)</td>
<td>1.540 (1.382–1.698)</td>
</tr>
<tr>
<td>Heart weight/body weight (g/kg)</td>
<td>4.31 (4.22–4.40)</td>
<td>4.36 (4.05–4.68)</td>
<td>3.71 (3.48–3.95)</td>
<td>3.68 (3.34–4.01)</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>0.930* (0.856–1.005)</td>
<td>1.243 (1.056–1.431)</td>
<td>1.853 (1.662–2.045)</td>
<td>1.759 (1.536–1.983)</td>
</tr>
<tr>
<td>Kidney weight/body weight (g/kg)</td>
<td>4.06* (3.79–4.32)</td>
<td>5.45 (4.67–6.23)</td>
<td>4.46 (4.05–4.87)</td>
<td>4.20 (3.73–4.66)</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>3.5† (3.2–3.7)</td>
<td>4.0 (3.6–4.3)</td>
<td>4.2 (3.9–4.5)</td>
<td>4.0 (3.6–4.4)</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td></td>
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</table>

All values are mean with the 95% confidence interval for the mean in parentheses. 
*p<0.05, **p<0.01 for significance tests by unpaired Student’s t test between control and transplanted SHR of each sex.

sex (MANOVA: females, $F_{1,15}=1.48, p=0.242$; males, $F_{1,15}=0.13, p=0.722$) or at the end of the experiment (Table 1). Transplanted males showed a slight fall in weight after the surgery but regained this deficit within a couple of weeks (data not shown).

Figure 1 shows the longitudinal blood pressure data, grouped after surgery according to the sex of the rat of origin of the kidney. All rats showed a gradual rise in systolic pressure with time, as seen in previous studies. The blood pressure of females whose kidneys had been replaced with those from male SHR remained significantly lower (MANOVA: $F_{1,15}=67.0, p<0.0001$) than the blood pressure of male uninephrectomy controls (Figure 1A). In fact, the blood pressure of transplanted females was slightly but significantly lower (MANOVA: $F_{1,15}=16.4, p=0.001$) than that of female uninephrectomy controls (see Figures 1A and 1B). Similarly, the blood pressure of males who had received female SHR kidneys remained significantly higher (MANOVA: $F_{1,15}=34.8, p<0.0001$) than that of female uninephrectomy controls (Figure 1B) and showed no tendency to fall as a result of transplantation. The blood pressures of male transplanted and control animals were not significantly different. Throughout the experiment the blood pressures of male controls were significantly higher than female controls by an average of 31 mm Hg (MANOVA: $F_{1,15}=51.2, p<0.0001$), and the difference between the transplanted male and female SHR was similar, being approximately 33 mm Hg (MANOVA: $F_{1,10}=83.1, p<0.0001$).

Mean arterial pressure recordings from conscious resting rats at 30 weeks of age confirmed the indirect systolic pressure data (Table 1), indicating no significant effect of transplantation in either sex. In addition, the heart weight and the heart weight/body weight ratio were not significantly different between the control and transplanted SHR of either sex (Table 1).

The transplanted kidneys were all found to be normal macroscopically and on sectioning showed no evidence of ureteric obstruction. The sizes of the transplanted kid-

Figure 1. Line graphs show preoperative (time zero) and postoperative (weeks 2–15) systolic blood pressure (mm Hg) of (panel a) rats that postoperatively had a single male kidney, either as result of transplantation into females (●) or uninephrectomy in males (○) and of (panel b) rats that postoperatively had a single female kidney, either as result of transplantation into males (●) or uninephrectomy in females (○). Values are mean±SEM; see text for statistical comparisons. SHR, spontaneously hypertensive rats.
The maintenance of the male-female blood pressure opposite sex once their own kidneys had been removed. Therefore, the possibility exists that the male and female SHR.

The kidney in the development and maintenance of SHR hypertension. Functional and hemodynamic abnormalities of the kidneys have been demonstrated in young SHR, and cross-breeding experiments have suggested that reduced glomerular filtration rate and renal plasma flow may be genetically determined abnormalities that cause high blood pressure. Transplantation of kidneys from male SHR increases blood pressure in animals with lower blood pressure, indicating an inherent capacity of male SHR kidneys to cause hypertension.

These issues may be relevant to mechanisms responsible for the dimorphism of SHR blood pressure have shown that the SHR Y chromosome increases blood pressure by both sex influence of autosomal genes and a distinct and separate hypertensive effect. Other studies of the X chromosome have demonstrated a locus at which SHR alleles code for lower blood pressure, 5-6 indicating an inherent capacity of male SHR kidneys to cause hypertension.

Previous cross-breeding and transplantation experiments have been made using male rats carrying the SHR Y chromosome, and it is unknown whether kidneys from female SHR exert an equivalent effect on blood pressure. Therefore, the possibility exists that the blood pressure difference between male and female SHR might have a renal basis determined by the Y chromosome. A simple and direct test of SHRs of both sexes should be the renal effects of sex chromosomes. Although these experiments are a general test of the renal effects of sex chromosomes, the negative outcome appears to exclude the possibilities that Y-linked genes increase or X-linked genes decrease blood pressure via intrinsic renal mechanisms in SHR. If genetic factors are important determinants of the transplantable hypertensive effects of SHR kidneys, our results suggest that they might be located on autosomal rather than sex chromosomes.

References

10. SPSS/PC+ for IBM PCXT/AT and Advanced Statistics. Chicago, III, MJ Norusis, SPSS Inc

Discussion

We found that neither the male nor the female SHR kidney altered the blood pressure of the recipient of the opposite sex once their own kidneys had been removed. The maintenance of the male-female blood pressure difference despite swapping kidneys implies that nonrenal factors are predominant and that the sexual dimorphism of SHR blood pressure is not the result of intrinsic renal differences. It is possible that the kidneys of male but not female SHR may express a sex-linked abnormality in early life that sets male pressure at a higher level but disappears in later life. However, this seems an unlikely explanation of our findings since the blood pressure effects of the transplanted male kidneys are the same when derived from young or old SHR.
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