Sodium homeostasis in transplanted rats with a spontaneously hypertensive rat kidney

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Received 19 April 1999; accepted in final form 10 May 2000

Frey, Bernd A. J., Olaf Grisk, Norman Bandelow, Siegfried Wussow, Peter Bie, and Rainer Rettig. Sodium homeostasis in transplanted rats with a spontaneously hypertensive rat kidney. Am J Physiol Regulatory Integrative Comp Physiol 279: R1099–R1104, 2000.—Recipients of a kidney from spontaneously hypertensive rats (SHR) but not from normotensive Wistar-Kyoto rats (WKY) develop posttransplantation hypertension. To investigate whether renal sodium retention precedes the development of posttransplantation hypertension in recipients of an SHR kidney on a standard sodium diet (0.6% NaCl), we transplanted SHR and WKY kidneys to SHR × WKY F1 hybrids, measured daily sodium balances during the first 12 days after removal of both native kidneys, and recorded mean arterial pressure (MAP) after 8 wk. Recipients of an SHR kidney (n = 12) retained more sodium than recipients of a WKY kidney (n = 12) (7.3 ± 10 vs. 4.0 ± 0.7 mmol, P < 0.05). MAP was 144 ± 6 mmHg in recipients of an SHR kidney and 106 ± 5 mmHg in recipients of a WKY kidney (P < 0.01). Modest sodium restriction (0.2% NaCl) in a further group of recipients of an SHR kidney (n = 10) did not prevent posttransplantation hypertension (MAP, 142 ± 4 mmHg). Urinary endothelin and urodilatin excretion rates were similar in recipients of an SHR and a WKY kidney. Transient excess sodium retention after renal transplantation may contribute to posttransplantation hypertension in recipients of an SHR kidney. Furthermore, adult SHR have a higher body sodium content than normotensive controls (22, 29).

We have recently shown that hypertension in genetically normotensive, bilaterally nephrectomized recipients of an SHR kidney is associated with excess renal sodium retention when the animals are subjected to a high-salt diet (20). In that study (20), measurements of daily sodium balances were started in the third week after renal transplantation when systolic blood pressure in recipients of an SHR kidney was already significantly elevated and the rats had just been transferred from a low-salt (0.2% NaCl) to a high-salt diet (1.8% NaCl). It remains unclear whether there is excess sodium retention in recipients of an SHR kidney vs. recipients of a WKY kidney without the challenge of a dietary salt load and, if there is excess sodium retention under these conditions, whether it precedes the development of hypertension.

The present study was designed to investigate the sodium homeostasis in renal transplanted rats with an SHR or WKY kidney on a diet that contained a standard (0.6% NaCl) or a low amount of sodium (0.2% NaCl) during a very early phase after transplantation and nephrectomy. In addition, we measured the urinary excretion of endothelin and urodilatin, two hormones that have been suggested to be involved in the regulation of renal sodium handling (8, 12, 13, 17).

METHODS

Animals. Experiments were performed with adult male normotensive WKY and SHR as kidney donors as well as with age-matched male F1 hybrids (F1H) bred from WKY and SHR parents as renal graft recipients. WKY and SHR were obtained at the age of 5 wk from Mollegaard (L. Skensved, Denmark). F1H were bred at the rat breeding facilities at the Ernst Moritz Arndt University Greifswald, Germany, from WKY and SHR parents that had been obtained from Mollegaard. Animals were housed in standard plastic cages or specially designed metabolic cages (see Experimental protocol) and maintained in a temperature- and humidity-controlled environment with lights on from 0600 h to 1800 h. If not otherwise indicated, animals had free access to standard chow food (Ssniff, Soest, Germany) and tap water.

IN RENAL TRANSPLANTATION EXPERIMENTS, hypertension travels with the kidney from spontaneously hypertensive rat (SHR) donors to normotensive recipients (20, 26, 27, 33, 34, 37), and kidneys from normotensive donors lower blood pressure in young transplanted SHR (32). The mechanisms underlying this phenomenon are currently not well understood but may be related to renal sodium handling. Thus SHR kidneys exhibit a rightward shift of their pressure-natriuresis curve compared with normotensive Wistar-Kyoto rats (WKY) (35, 36). Young SHR retain more sodium during the early developmental phase of hypertension than age-matched WKY (2, 23), which also are in positive sodium balance as long as they gain body weight.
ad libitum. All experiments were approved by the governmental committee on animal welfare of the state of Mecklenburg-Vorpommern, Germany.

Surgery. Surgery for nephrectomy, renal transplantation, and implantation of femoral artery catheters was performed as previously described in detail (27, 37). Briefly, both donor and recipient were anesthetized with ketamine (Ketamin 10%, cp-pharma, Burgdorf, Germany), 100 mg/kg body wt ip, plus xylazine (Rompun; Bayer, Leverkusen, Germany), 10 mg/kg body wt ip, and simultaneously operated on by two investigators. During surgery, ketamine was supplemented as needed. The kidney, including the renal artery with a short piece of aorta, the renal vein with a patch of the vena cava, and the whole ureter were removed from the donor and immediately transferred to the recipient, which at that time was ready to receive the graft. An end-to-side anastomosis was performed between the donor and the recipient aorta. The renal vein was anastomosed end-to-side to the recipient vena cava. The ureter was placed into the bladder through a small incision in the bladder wall at the apex. During surgery, the graft was wrapped in gauze and repeatedly rinsed with ice-cold isotonic saline. Total ischemia time was always less than 60 min.

Blood pressure measurements. Direct recordings of arterial blood pressure in conscious unrestrained rats were performed as previously described (32). Two days prior to arterial pressure recordings, catheters (PE-10 fused to PE-50) were inserted into the aorta via the left femoral artery and exteriorized at the back of the neck. The catheters were filled with isotonic saline containing 250 IU/ml heparin and plugged. On the day of the experiment, catheters were connected to an Isotec pressure transducer coupled to a direct current bridge amplifier (type 660; Hugo Sachs Elektronik, March-Hugstetten, Germany) and a linear recorder (model WR 3300; Hugo Sachs Elektronik). Blood pressure was recorded over a 60-min period between 8 AM and 10 AM with the animals resting or grooming in their home cage.

Sodium concentration. Sodium concentrations in plasma and urine were measured with ion-selective membrane electrodes (AVL ISE Analysator; AVL List, Graz, Austria). Sodium concentrations in food and feces were determined by atomic absorption spectrometry (Solaar 939; Unicam, Cambridge, UK). Briefly, food or feces samples were dissolved in concentrated sulfuric acid (Merck, Darmstadt, Germany) at a ratio of 40 ml sulfuric acid per gram sample. After storage at room temperature for at least 24 h, the solution was stirred and heated to 200°C. Hydrogen peroxide (Merck) was added until the solution became clear. The solution was allowed to cool down to room temperature, adjusted to a predetermined volume by addition of sodium-free distilled water, and subjected to determinations of sodium concentration by atomic absorption spectrometry.

Endothelin and urodilatin. The urinary concentrations of endothelin and urodilatin were determined by radioimmunoassay after extraction. The extraction procedure has been described elsewhere (5). Briefly, acidified samples (4% acetic acid) were put on a preconditioned (4% acetic acid in 96% ethanol, 100% methanol, water, and 4% acetic acid) C_{18} Sep-Pak cartridge (Waters, Millipore, Bedford, MA). After washing the column with water, peptides were eluted with 4% acetic acid in 60% ethanol. The eluate was evaporated to dryness, and the extracts were stored at −18°C. For radioimmunoassay, extracts were redissolved in assay buffer (0.1 mol/l phosphate buffer with 0.01 mol/l dipotassium EDTA and 1.0 g/l human serum albumin, pH 7.4).

The concentration of endothelin-1 in urine was determined using a specific antibody (RAS 6901) purchased from Peninsula Laboratories (St. Helens, Merseyside, UK). The procedure has been described previously (11). The detection limit was about 0.4 pg/ml, and the mean extraction recovery of unlabeled endothelin-1 added to plasma was 90%. Intra- and interassay coefficients of variation were 5% and 7%, respectively.

The concentration of uroladin in urine was determined using a highly specific antibody (S 1969; kindly supplied by Biomedica, Vienna, Austria) as recently described (4). The detection limit was 0.32 pg/ml of urine, and mean extraction recovery of unlabeled urodilatin added to urine was 85%. The intra-assay coefficient of variation was 10%.

Experimental protocol. F1H were randomly assigned to three groups. Group 1 (n = 12) received a WKY kidney, whereas groups 2 (n = 12) and 3 (n = 10) received an SHR kidney. At the time of transplantation, F1H recipients were 8–9 wk old and had similar body weights (group 1, 200 ± 7 g; group 2, 212 ± 9 g; and group 3, 192 ± 7 g, not significant [NS]). Donors were of the same age and had similar body weights (group 1, 195 ± 14 g; group 2, 211 ± 11 g; and group 3, 184 ± 10 g, NS). The left native kidney of the recipients was removed while they were under anesthesia for renal transplantation. The remaining native kidney was removed 1 wk later. The day of removal of the second native kidney was defined as day 0 of the protocol.

After recovery from anesthesia for renal transplantation, the recipients were transferred to metabolic cages where they remained for 19 days. While they were in metabolic cages, animals were fed a sodium-free granular rat chow (Sniff) that was supplemented with a known amount of sodium chloride to result in a NaCl content of 0.6% (groups 1 and 2) and 0.2% (group 3), respectively. The sodium content of the diet was verified by regular measurements. During the metabolic study, animals had access to distilled water ad libitum. Every day, a known amount of chow was offered to each rat in a special trough. The trough was equipped with a food trap to minimize spillage. After each 24-h period, food remaining in the trough or the food trap was weighed and daily sodium intake was calculated. Twenty-four-hour urine and feces samples were quantitatively collected from each rat in separate volumetric cylinders. Urine volume and weight of feces were recorded, and samples were stored at −20°C. Total daily sodium excretion was determined as the sum of daily urinary and fecal sodium excretion.

At the end of the metabolic study, a blood sample (1 ml) was withdrawn from the retroorbital plexus under anesthesia, and the animals were returned to standard rat cages. Eight weeks after renal transplantation, a catheter was implanted into the right femoral artery, tunneled under the skin, and exteriorized at the scuff of the neck. The catheter was filled with isotonic saline containing 200 IU/ml heparin. After placement of the catheter, animals were allowed to recover for 1 wk before direct blood pressure measurements were performed.

Statistics. Results are expressed as means ± SE. Data were analyzed by one-way ANOVA or by two-way ANOVA (factors group and time) with repeated measurements on one factor (time) as appropriate. When appropriate, a Bonferroni post hoc test was performed. Statistical significance was accepted at P < 0.05.

RESULTS

Following the removal of both native kidneys in two steps, renal transplanted (WKY × SHR)-F1H on a diet containing 0.6% and 0.2% NaCl, respectively, rapidly recovered from surgery and gained weight. The
amount of body weight gained within 12 days starting at the day of removal of the second native kidney, i.e., 7 days after renal transplantation and removal of the first native kidney, was $33 \pm 10$ g in group 1, $46 \pm 4$ g in group 2, and $45 \pm 5$ g in group 3. The differences between groups were not statistically significant ($P = 0.32$).

Daily sodium intake and excretion were significantly less in rats maintained on a low-sodium diet than in rats on a diet containing a standard amount of sodium (Fig. 1). There were also statistically significant differences in sodium intake between recipients of a WKY and an SHR kidney on the standard diet on days 6, 8, 10, and 11 of the protocol. All three groups showed a positive cumulative sodium balance over 12 days following the removal of the second native kidney. During this time, recipients of a WKY kidney on a 0.6% NaCl diet retained a total amount of $7.3 \pm 1.0$ mmol sodium, whereas recipients of an SHR kidney on a 0.6% NaCl diet retained only $4.0 \pm 0.7$ mmol sodium. Recipients of an SHR kidney on a 0.2% NaCl diet retained $5.2 \pm 0.9$ mmol sodium. An overall ANOVA revealed statistically significant within- ($P < 0.001$) and between-subjects differences ($P < 0.05$) as well as a significant group $\times$ time interaction ($P < 0.001$). Post hoc analyses showed that cumulative sodium retention was significantly higher in recipients of an SHR kidney on a 0.6% NaCl diet than in recipients of a WKY kidney on the same diet on days 9 through 12 of the protocol. Plasma sodium concentrations were $143 \pm 3$ mmol/l in recipients of a WKY, $144 \pm 6$ mmol/l in recipients of an SHR kidney on a standard sodium diet, and $143 \pm 3$ mmol/l in recipients of an SHR kidney on a low-salt diet ($P = 0.79$).

Daily urinary endothelin excretion rates (Fig. 2A) followed different patterns in recipients of a WKY kidney and an SHR kidney, respectively. Immediately after removal of the second native kidney, urinary endothelin excretion was higher in recipients of a WKY kidney than in recipients of an SHR kidney. Subsequently, urinary endothelin excretion rates decreased in recipients of a WKY kidney to the level of that in
recipients of an SHR kidney. Minor and less consistent fluctuations were observed in recipients of an SHR kidney. An overall statistical analysis by two-way repeated-measurements ANOVA revealed no significant effects of the factors group \((P = 0.30)\) and time \((P = 0.25)\), but a significant group/time \((P < 0.01)\) interaction. Daily urinary urodilatin excretion rates (Fig. 2B) were not different between the two groups \((P = 0.63)\), but there was a statistically significant decrease with time \((P < 0.01)\).

Eight weeks after renal transplantation, directly measured arterial pressures (Fig. 3) were significantly higher in recipients of an SHR kidney than in recipients of a WKY kidney on a standard sodium diet \((P < 0.01)\). Modest sodium restriction did not prevent the development of posttransplantation hypertension in recipients of an SHR kidney.

**DISCUSSION**

An important mechanism by which the kidney contributes to the regulation of long-term blood pressure is renal sodium handling \((9, 21)\). SHR kidneys require higher perfusion pressures than WKY kidneys to excrete the same amount of sodium \((35, 36)\). The rightward shift of the pressure-natriuresis curve in SHR vs. WKY kidneys appears to be intrinsic to the kidney, since it was preserved when differences in neural and endocrine influences were minimized by renal denervation and by maintaining plasma levels of vasopressin, aldosterone, cortisol, and norepinephrine constant \((35, 36)\). In keeping with these findings, young prehypertensive SHR have been shown to exhibit transient excess sodium retention during the developmental phase of hypertension \((2, 22)\), and it has been suggested that this mechanism may be a causal factor for the development of hypertension in this strain \((9, 21)\).

We have previously shown that recipients of an SHR kidney have a decreased ability to excrete sodium when challenged with a high-salt diet \((20)\). This finding is compatible with the hypothesis that a shift in the pressure-natriuresis curve of SHR kidneys is the primary mechanism for the development of hypertension in recipients of a kidney from this strain. In that study \((20)\), rats were in the chronic phase after transplantation and blood pressure was already higher in recipients of an SHR kidney than in recipients of a WKY kidney when metabolic measurements began. Furthermore, rats were fed a high-salt diet. It remained therefore unclear whether recipients of an SHR kidney would also retain more sodium than recipients of a WKY kidney when they are maintained on a diet containing a standard amount of sodium \((0.6\% \text{NaCl})\) and, if yes, whether sodium retention precedes the development of posttransplantation hypertension.

In the present study metabolic measurements commenced on the day of removal of the second native kidney, i.e., 7 days after removal of the first native kidney and renal transplantation. At that time, blood pressure in adult normotensive recipients of an SHR kidney is not yet significantly elevated \((20, 33, 34, 37)\). The present results show that there is increased sodium retention in recipients of an SHR kidney vs. recipients of a WKY kidney within 12 days after renal transplantation and bilateral nephrectomy when the rats are maintained on a diet containing a standard amount of sodium. In other words, increased sodium retention in recipients of an SHR kidney vs. recipients of a WKY kidney on a standard sodium diet precedes the development of hypertension in the former group. Although it is not direct evidence, this finding suggests that there may be a cause-and-effect relationship between sodium retention and hypertension in recipients of an SHR kidney.

On the other hand, recipients of an SHR kidney on a low-sodium diet also developed posttransplantation hypertension, yet cumulative sodium balance within 12 days after removal of the second native kidney was not significantly different from that of recipients of a WKY kidney on a standard sodium diet. It should be noted, however, that there was a tendency, albeit not statistically significant, for recipients of an SHR kidney on a low-sodium diet to retain more sodium than recipients of a WKY kidney on a standard sodium diet. Since our study was designed to investigate sodium homeostasis in the early phase after renal transplantation and removal of both native kidneys, we do not have information on sodium balances beyond this period. The slopes of the cumulative sodium balance curves and the finding that the differences in cumulative sodium balance between recipients of a WKY and SHR kidney on a standard sodium diet did not become statistically significant before the last 4 days of our observation period suggest that we may have missed a true difference in sodium retention between recipients of an SHR kidney on a low-sodium diet and recipients of a WKY kidney on a standard sodium diet, which may have preceded the development of posttransplantation hypertension in the former group.

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**Fig. 3. Directly measured systolic and diastolic blood pressures in renal transplanted rats. \(^*P < 0.01\) vs. recipients of a WKY kidney.**
Clearly, the reduction of the sodium content in the diet to about one-third of the usual amount in ordinary rat chow did not prevent nor attenuate posttransplantation hypertension. The sodium concentration in our low-sodium diet was chosen on the basis of reports (15, 16) that a diet containing less than about 3.5 mmol sodium per 100 g food (=0.2% NaCl) causes homeostatic disturbances such as salt hunger and reduced tolerance to blood loss, to which SHR are particularly sensitive (10, 19). Our finding that recipients of an SHR kidney on a low-sodium diet retained at least as much sodium as recipients of a WKY kidney on a standard sodium diet illustrates the strong propensity of transplanted SHR kidneys to retain sodium.

During the study period, there were subtle differences between the groups in sodium intake and excretion. The reasons for the slight differences in sodium intake between the two groups of F1H recipients are not obvious to us. It is important to note, however, that transplanted SHR kidneys excreted a smaller fraction of the consumed sodium than transplanted WKY kidneys resulting in excess sodium retention in the former group. Recipients of an SHR kidney exhibited a lower rate of urinary endothelin excretion than recipients of a WKY kidney immediately after removal of the second native kidney. It has been shown that urinary endothelin is of renal origin rather than being derived from the plasma (1, 3, 38). It has also been shown that renal endothelin production is reduced in adult SHR compared with WKY (25). Since endothelin has potent natriuretic actions that are independent of its hemodynamic effects (18, 24, 38), it has been postulated that the decreased renal endothelin production in SHR kidneys may contribute to inappropriate sodium retention (25). Our data that urinary endothelin excretion in transplanted rats immediately after removal of the second native kidney was lower in recipients of an SHR kidney than in recipients of a WKY kidney are compatible with this notion. On the other hand, the initial difference in urinary endothelin excretion between recipients of a WKY kidney and recipients of an SHR kidney disappeared rapidly, resulting in similar excretion rates during most of the protocol, whereas sodium retention continued to be different. In addition to its potential role in renal sodium handling, kidney-derived endothelin has been implicated in several pathophysiological states (6, 8) including renal ischemia (14, 30) and chronic renal failure (3, 7, 31). Furthermore, it has recently been shown that unilateral nephrectomy increases endothelin-1 mRNA in the remaining kidney (28). Although there is no evidence for renal failure in the present study, the transplanted kidneys had been subjected to cold ischemia during transplantation surgery, and the grafts functioned as solitary kidneys. Thus urinary endothelin excretion in the present model may have been affected by several different mechanisms possibly related to the regulation of sodium handling.

Urinary urodilatin excretion rates were not significantly different between recipients of a WKY and an SHR kidney. Urodilatin has so far only been found in the kidney, where it is supposed to be involved in the regulation of sodium excretion (17). Our data do not support a role for urodilatin in sodium retention in recipients of an SHR kidney.

Taken together, the present data indicate that hypertension in recipients of an SHR kidney on a standard rat diet is preceded by excess renal sodium retention. Lowering the sodium content in the diet to about one-third of that in ordinary rat chow does not attenuate posttransplantation hypertension. Renal endothelin and urodilatin probably do not play a major role in the development of hypertension in this model. Transient excess sodium retention after renal transplantation may contribute to posttransplantation hypertension in recipients of an SHR kidney.

Perspectives

Renal transplantation studies using several genetically hypertensive rat strains showed that arterial hypertension travels with the kidney. The mechanisms underlying this phenomenon have not yet been identified. Our data suggest that increased renal sodium retention may play a role in bilaterally nephrectomized recipients of an SHR kidney. The mechanisms involved in exaggerated sodium retention during the development of renal posttransplantation hypertension remain to be investigated. The experimental approach of kidney cross-transplantation between histocompatible rat strains with a varying degree of genetic predisposition to hypertension allows one to determine the contribution of renal mechanisms to elevated arterial pressure. Future experiments designed to manipulate intrarenal and extrarenal mechanisms involved in kidney development during early ontogeny in kidney donors will provide an additional approach to investigate the contribution of renal mechanisms to arterial hypertension.

We thank Doreen Block and Brigitte Sturm for expert technical assistance.

This study was supported by Deutsche Forschungsgemeinschaft Grant Re 522/7-2, by the Danish Medical Research Council, and by EU Grant ERBCHRXT-930645.

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