Sympathetic-renal interaction in chronic arterial pressure control

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Grisk, Olaf, Hans-Joachim Rose, Gerd Lorenz, and Rainer Rettig. Sympathetic-renal interaction in chronic arterial pressure control. Am J Physiol Regulatory Integrative Comp Physiol 283: R441–R450, 2002. First published May 6, 2002; 10.1152/ajpregu.00669.2001.—The effects of neonatal sympathectomy of donors or recipients on post-transplantation arterial pressure were investigated in spontaneously hypertensive rats (SHR) by renal transplantation experiments. Conscious mean arterial pressure (MAP) and renal vascular resistance were 136 ± 1 mmHg and 15.5 ± 1.2 mmHg·ml⁻¹·min⁻¹ in sympathectomized SHR (n = 8) vs. 158 ± 4 mmHg (P < 0.001) and 20.8 ± 1.1 mmHg·ml⁻¹·min⁻¹ (P < 0.05) in controls (n = 10). Seven weeks after transplantation of a kidney from neonatally sympathectomized SHR donors, MAP in SHR recipients (n = 10) was 20 mmHg lower than in controls transplanted with a kidney from hydralazine-treated SHR (n = 10) (P < 0.05) associated with reduced sodium sensitivity of MAP. Neonatal sympathectomy also lowered MAP in F1-hybrids (F1H; SHR × Wistar-Kyoto rats). Within 6 wk after transplantation, renal grafts from untreated SHR increased MAP by 20 mmHg in sympathectomized F1H (n = 10) and by 35 mmHg in sham-treated F1H (n = 8) (P < 0.05). Neonatal sympathectomy induces chronic changes in SHR kidney function leading to a MAP reduction even when extrarenal sympathetic tone is restored. Generalized reduction in sympathetic tone resets the kidney-fluid system to reduced MAP and blunts the extent of arterial pressure rise induced by an SHR kidney graft.

Spontaneously Hypertensive rats (SHR) of the Okamoto-Aoki strain show an increased density of sympathetic target organ innervation compared with normotensive rats (17). Functionally, the sympathetic nervous system of SHR is characterized by elevated effenent peripheral nerve activity (20, 25) and enhanced reactivity to environmental stimuli compared with normotensive rats (18, 25). The question arises whether there is a cause and effect relationship between elevated sympathetic activity and hypertension in SHR.

Support for a major role of the sympathetic nervous system in primary hypertension in SHR has come from studies showing a long-term arterial pressure reduction in SHR after neonatal destruction of peripheral sympathetic nerves (10, 23, 24). The hypertensive effect of neonatal sympathectomy in SHR is associated with a decreased hindlimb vascular resistance (10, 23) and a reduced mesenteric arterial wall/lumen ratio (24).

On the other hand, the fact that transplanted and therefore denervated kidneys from SHR donors cause hypertension in normotensive recipients (15) suggests that long-term arterial pressure in SHR may be primarily determined by mechanisms related to the kidney rather than the sympathetic nervous system (16). This notion is supported by the finding that arterial pressure can be dramatically reduced in SHR after transplantation of a kidney from normotensive histocompatible donors (14). Renal and sympathetic nervous system mechanisms are not mutually exclusive. Elevated effenent renal sympathetic nerve activity can contribute to hypertension by increasing renal vascular resistance (RVR), renin release, and renal tubular sodium reabsorption (8). Evidence for a role of sympathetic renal innervation for the development of hypertension in SHR has been provided by renal denervation experiments (33). Renal denervation in 7-wk-old SHR delays hypertension development by ~4 wk, whereas renal denervation in 18-wk-old SHR has no effect on arterial pressure (33).

The degree to which a reduction in sympathetic tone affects long-term arterial pressure in SHR appears to depend on the age at which sympathetic denervation is performed and on the extent of sympathetic denervation, i.e., renal denervation (33) vs. generalized reduction in sympathetic tone (10, 23, 24). The early removal of sympathetic tone to the kidney may be particularly important. To specifically investigate the influence of early postnatal sympathetic tone on the ability of an SHR kidney to maintain arterial hypertension, we chose to transplant a kidney from neonatally sympathectomized SHR into untreated SHR. Arterial pressure was measured telemetrically, and sodium sensitivity of arterial pressure was determined. In an attempt to investigate underlying mechanisms, we
measured effects of neonatal sympathectomy on RVR in SHR.

In previous experiments, we demonstrated that development of renal posttransplantation hypertension in recipients of a kidney graft from SHR does not depend on specific activation of the sympathetic nervous system (13). However, the influence of sympathetic tone on the extent of the arterial pressure rise induced by an SHR kidney graft and the absolute level of renal posttransplantation hypertension remains unknown. Therefore, we investigated if a generalized reduction in sympathetic tone modifies hypertensive actions of SHR kidney grafts obtained from untreated young adult animals.

METHODS

Animals. Experiments were performed in male SHR and in male F1-hybrids (F1H) obtained from breeding male SHR and female Wistar-Kyoto rats (WKY). Male and female SHR for breeding SHR and F1H were obtained from M&B A/S (Ry, Denmark). Female WKY for breeding F1H were obtained from Charles River (Sulzfeld, Germany). Animals for experimentation were bred in our institution’s animal facility. Animals were kept in a humidity- and temperature-controlled environment with lights on from 6 AM to 6 PM. If not stated otherwise, they had free access to standard rat chow containing 0.25% sodium (Ssniff, Soest, Germany) and tap water. All experiments were approved by a governmental committee on animal welfare.

Neonatal sympathectomy. Sympathectomy was performed in neonatal SHR and F1H by daily intraperitoneal injections of guanethidine (guanethidine monosulfate, Sigma-Aldrich Chemicals, Steinheim, Germany) at a dose of 50 μg/g body wt between postnatal days 5 and 28 (19). The drug was dissolved in isotonic saline and pH was adjusted to 7.4. Controls received isotonic saline injections during the respective time interval. Animals were weaned on postnatal day 28. One day after weaning, the adrenal medulla was bilaterally removed in guanethidine-treated animals. Adrenals were approached via flank incisions under ether anesthesia, and a small incision was made in the adrenal cortex with a cannula. The adrenal medulla was removed by gently squeezing the adrenals with cotton-tipped applicators. Control animals were sham operated.

Animal instrumentation. For arterial pressure recordings, catheters (PE-10 fused to PE-50) were inserted into the inferior abdominal aorta via the left or right femoral artery and exteriorized and fixed at the back of the neck. The catheters were filled with isotonic saline containing 250 IU/ml heparin and plugged. For measurements of renal blood flow (RBF), animals were implanted with ultrasound transit time flow probes (1RB, Transonic Systems, Ithaca, NY) under ether anesthesia. Flow probes were placed around the left renal artery through a retroperitoneal approach. After closure of the left flank incision, cable and connector of the probe were exited at the back of the neck and fixed with a saddleback cuff. For telemetric arterial pressure recordings, renal transplanted animals were implanted with telemetric devices (TA11PA-C40, Data Sciences International, St. Paul, MN). Implantation was performed immediately after completion of right nephrectomy. Before implantation, the caudal abdominal aorta was freed from surrounding connective tissue, and blood flow below the renal graft anastomosis was interrupted by vessel clips. Catheters of telemetric devices were inserted into the aorta and glued with veterinary adhesive. Thereafter, vessel clips were released, the abdomen was closed in layers, and the telemetric device was sutured to the abdominal wall.

Renal transplantation. Kidney transplantation was performed as described previously (13–15). Donor and recipient were operated simultaneously by two investigators. Before transplantation, the recipient’s left kidney was excised. Cold ischemia time of the grafts did not exceed 45 min. The graft was anastomosed to the abdominal aorta with an aortic patch obtained from the donor to prevent stenosis of the grafted renal artery. The renal vein of the graft was sutured end to side to the inferior vena cava of the recipient. Seven days after transplantation, the recipient’s right native kidney was excised.

Metabolism experiments. To study the relationship between sodium intake, renal sodium excretion, and arterial pressure, animals instrumented with telemetric devices for arterial pressure recording were placed into plastic metabolism cages with the receivers immediately behind. Arterial pressure, sodium intake, and renal sodium excretion were monitored continuously allowing for construction of renal function curves when the content of dietary sodium chloride was changed (16, 21). Animals were fed a pulverized diet (Ssniff) containing either 22 mol NaCl/g food (0.12% NaCl) or 300 mol NaCl/g food (1.8% NaCl). The trough was equipped with a cup that prevented spillage. Urine was collected separately from feces under mineral oil (14). The relationship between mean arterial pressure (MAP) and urinary sodium excretion (UNaV) was assumed to be linear (21). UNaV was expressed as a first-order function of MAP: UNaV = B (MAP – A) (21). A is the intercept of the renal function curve with the abscissa (MAP axis), and B is the slope of the renal function curve. The reciprocal of B is a measure of sodium sensitivity of arterial pressure (21).

Analytic methods. For measurements of renal and adrenal catecholamine concentrations, kidneys and adrenals were rapidly removed from animals under deep ether anesthesia, blotted, weighed, and snap-frozen in isopentane and liquid nitrogen. Tissues were homogenized for 1 min at maximum speed in 10 ml ice-cold perchloric acid (0.2 mol/l). After centrifugation, the clear supernatant was stored at −70°C until catecholamine extraction and measurement. For measurement of plasma catecholamine concentrations, 2 ml of blood were sampled from arterial catheters in quietly resting animals after termination of the final direct arterial pressure recordings. The blood was drawn into prechilled heparinized tubes. After centrifugation at 4°C, plasma samples were stored at −70°C until catecholamine extraction and measurement. Catecholamines were extracted by adsorption to aluminum oxide at pH 8.6 using a commercially available kit (Recipe Chemicals and Instruments, Munich, Germany). After being washed three times with distilled water, catecholamines were released from aluminum oxide with perchloric acid (0.5 mol/l). Norepinephrine and epinephrine were measured by reverse-phase high-performance liquid chromatography with an integrated detector for reduction and oxidation (Intro, Antec, Leyden, The Netherlands). Oxidation potential was ±600 mV (15).

Urinary sodium concentration was measured with an ion-selective electrode (AVL, Graz, Austria). Urinary protein concentration was measured by the pyrogallol red method (Biocon, Lichtenfels, Germany). Plasma creatinine and urea concentrations were measured photometrically (Kreatinin liquicolor and Harnstoff liquicolor, Human, Taunusstein, Germany).

Histology. Coronal slices from transplanted kidneys including cortex and medulla were immersion fixed in 4%
buffered formaldehyde. After fixation, tissue samples were embedded in paraffin and cut. Slices were stained with hematoxylin-eosin. To visualize elastic fibers, staining was performed with resorcinol and fuchsin. Morphological evaluation was performed by a pathologist (G. Lorenz) who was blinded for the experimental design. The severity of glomerular, tubular, interstitial, and vascular lesions was graded according to a simplified score with 0 = no lesions, 1 = slight lesions, 2 = intermediate lesions, and 3 = severe lesions.

**Data acquisition.** For arterial pressure recordings, catheters were connected to a pressure transducer (Isotec, Hugo Sachs Elektronik, March, Germany). The pressure signal was amplified with a DC bridge amplifier (Hugo Sachs Elektronik). Heart rate was derived from the pulsatile arterial pressure signal. Flow probes were connected to an ultrasound transit time flowmeter (Transonic). The amplified signals were fed into a personal computer, digitized at 500 Hz, and displayed and analyzed with “Universal Acquisition” software version 7 kindly provided by Drs. M. A. Navakatikyan and S. C. Malpas, University of Auckland, New Zealand. The software is based on LabView graphical programming language (National Instruments, Austin, TX).

Data acquisition of telemetric arterial pressure recordings was performed with Dataquest LabPro version 3.11 (Data Sciences International). Data were sampled every 10 min at 500 Hz for 10 s. When animals were kept in individual standard cages, 24-h recordings were performed on every other day. During metabolism experiments, recordings were performed throughout the entire period.

**Experimental protocol 1.** Three groups of rats were differentially treated in preparation for transplantation experiments. SHR (n = 7) were neonatally sympathectomized, sham sympathectomized (n = 5), or sham sympathectomized and treated with hydralazine (Novartis Pharma, Wehr, Germany) 50 mg·kg⁻¹·day⁻¹ in the drinking water, beginning immediately after weaning (n = 5). At the age of 12 wk, animals were implanted with intra-arterial catheters, and arterial pressure was recorded 2 days later. Thereafter, kidneys and adrenals from sympathectomized and sham-sympathectomized, hydralazine-treated animals were removed for measurement of tissue catecholamine contents.

**Experimental protocol 2.** For measurement of arterial pressure, RBF, and for calculation of RVR, neonatally sympathectomized SHR (n = 8) and sham-sympathectomized SHR (n = 10) were implanted with an arterial catheter and an ultrasound transit time flow probe at the age of 12 wk. Animals were allowed to recover for 2 days. Simultaneous recordings of arterial pressure and RBF were performed in conscious unrestrained animals in their home cage. Recordings were performed over 30 min. After completion of recordings, left kidneys were removed under deep ether anesthesia, blotted, and weighed.

**Experimental protocol 3.** Two groups of 9- to 10-wk-old male untreated SHR (n = 10/group) were transplanted either with a kidney from neonatally sympathectomized or sham-sympathectomized and hydralazine-treated SHR. The left native kidney was removed. Kidney donors were age matched to the recipients. After a recovery period of 7 days, right native kidneys were excised, and animals were implanted with a telemetry device for arterial pressure recording. After another 7 days of recovery, telemetric arterial pressure recordings were started for 2 wk with the animals in individual standard cages with free access to food and water. Thereafter, animals were transferred into metabolism cages and offered a low-NaCl diet (22 µmol NaCl/g food). Animals were allowed to adapt to the new environment and the low-NaCl diet for 3 days. On day 34, the diet was changed to a high-NaCl diet containing 300 µmol NaCl/g food. Within 24 h, sodium intake and renal sodium excretion reached a new steady state. For construction of renal function curves, the respective data on MAP and UNaV obtained during experimental days 30-33 (low-NaCl diet) and 35-38 (high-NaCl diet) were averaged. After termination of metabolism experiments, animals were transferred back into standard cages with free access to food and water. Animals were allowed to adapt for 24 h, and arterial pressure recordings were continued for another 5-day period. At the end of the experiments, animals were deeply anesthetized with ether, and the grafted kidneys were removed for histological examination.

**Experimental protocol 4.** Neonatally sympathectomized F1H (n = 11) and sham-sympathectomized F1H (n = 8) were implanted with an arterial catheter via the left femoral artery between 9 and 10 wk of age. Two days later, direct arterial pressure recordings were performed in conscious unrestrained animals. Thereafter, the catheters were removed from the animals, the wounds were closed in layers, and each group was transplanted with a kidney obtained from age-matched, untreated SHR. Both native kidneys were removed as described above. Six weeks after renal transplantation, animals were implanted with arterial catheters via the right femoral artery, and arterial pressure was recorded in conscious animals 2 days later. When recordings were finished, a blood sample was drawn from the arterial catheter for measurement of plasma catecholamine, urea, and creatinine concentrations. Then, animals were deeply anesthetized, and the kidneys were removed for histological examination.

**Statistics.** Comparisons of group means were performed with unpaired t-test or by one-way analysis of variance as appropriate. Comparisons of group means with repeated measurements were performed by two-way analysis of variance followed by Student-Newman-Keuls test to identify differences between individual group means when significant effects of treatment, time, or interactions of both were found. Differences were taken as significant at P < 0.05. Data in text, tables, and figures are presented as means ± SE.

**RESULTS**

**Effectiveness of neonatal sympathectomy.** Neonatal sympathectomy resulted in a reduction of MAP by ~30 mmHg in 12-wk-old SHR compared with sham-treated controls (Fig. 1). Hydralazine treatment lowered arterial pressure to a similar degree. Systolic arterial pressure (SAP) and diastolic arterial pressure (DAP) were reduced by one order of magnitude, and adrenal catecholamine concentrations were reduced by two orders of magnitude compared with hydralazine-treated animals (Fig. 1). Left kidney weight was 0.81 ± 0.03 g in sham-treated animals, 0.79 ± 0.01 g in hydralazine-treated animals, and 0.86 ± 0.02 g in sympathectomized animals, respectively (not significant). Kidney weight-to-body weight ratio (g/100 g body wt) was 0.31 ± 0.01 in sham-treated SHR, 0.33 ± 0.01 in hydralazine-treated SHR, and 0.36 ± 0.01 in sympathectomized SHR, respectively (sham-treated vs. sympathectomized SHR; P < 0.05).
RVR. At the age of 12 wk, RBF was similar in neonatally sympathectomized and sham-sympathectomized SHR. SAP, MAP, and DAP were significantly lower in sympathectomized rats, resulting in reduced RVR compared with sham-sympathectomized SHR (Table 1).

Effect of kidney grafts from sympathectomized donors on arterial pressure in untreated SHR. During the third and fourth week after renal transplantation, MAP did not differ significantly between recipients of a kidney from sympathectomized donors and recipients of a kidney from hydralazine-treated donors. There was a trend toward higher values in the latter group (Fig. 2). When animals were exposed to a low-NaCl diet followed by a high-NaCl diet, 24-h values on sodium intake and natriuresis (UNaV) did not differ between groups (Fig. 3). Water intake and diuresis were similar in both groups (data not shown). After the switch to a high-NaCl diet, body weight rose from 286 ± 7 to 311 ± 7 g in recipients of a sympathectomized kidney and from 286 ± 8 to 302 ± 8 g in recipients of a hydralazine-pretreated kidney within 5 days. There was no significant difference in the rise of body weight between both groups. During the challenge with different amounts of dietary sodium, arterial pressure showed a greater rise in recipients of a kidney from hydralazine-pretreated donors than in recipients of a kidney from sympathectomized animals (Figs. 2 and 4). Sodium sensitivity of arterial pressure as measured by the inverse slope of renal function curves was lower in recipients of a kidney from sympathectomized donors than in recipients of a kidney from hydralazine-treated donors (Fig. 4). The extrapolated intercepts of the renal function curves with the MAP axis (MAP at UNaV = 0) were 141 ± 2 mmHg in recipients of a kidney from sympathectomized donors and 150 ± 5 mmHg in recipients of a kidney from hydralazine-treated donors, respectively (not significant). During the last week of the protocol with the animals on standard rat chow and in standard cages on wooden bedding, MAP was significantly lower in recipients of a kidney from sympathectomized donors than in recipients of a kidney from hydralazine-treated donors (Fig. 4). The extrapolated intercepts of the renal function curves with the MAP axis (MAP at UNaV = 0) were 141 ± 2 mmHg in recipients of a kidney from sympathectomized donors and 150 ± 5 mmHg in recipients of a kidney from hydralazine-treated donors, respectively (not significant).

Table 1. Arterial pressure, RBF, RVR, body weight, and kidney weight in 12-wk-old neonatally sympathectomized and sham-sympathectomized SHR

<table>
<thead>
<tr>
<th></th>
<th>Sympathetomized SHR (n = 8)</th>
<th>Sham-Sympathectomized SHR (n = 10)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAP, mmHg</td>
<td>158 ± 4</td>
<td>184 ± 6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>136 ± 1</td>
<td>158 ± 4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DAP, mmHg</td>
<td>120 ± 4</td>
<td>133 ± 4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>*RBF, ml·min⁻¹·g⁻¹</td>
<td>8.8 ± 1</td>
<td>7.6 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>*RVR, mmHg·ml⁻¹·min⁻¹</td>
<td>15.5 ± 1.2</td>
<td>20.8 ± 1.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>254 ± 4</td>
<td>271 ± 4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Left kidney weight, g</td>
<td>0.84 ± 0.02</td>
<td>0.89 ± 0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Kidney weight/body weight, g/100 g</td>
<td>0.33 ± 0.01</td>
<td>0.33 ± 0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Data are related to 1 g wet kidney wt. RBF, renal blood flow; RVR, renal vascular resistance; SHR, spontaneously hypertensive rats; SAP, systolic arterial pressure; MAP, mean arterial pressure; DAP, diastolic arterial pressure; NS, not significant.

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Renal damage in grafts from sympathectomized and hydralazine-treated animals. Plasma creatinine and urea concentrations measured at the end of the experiments did not indicate major differences in renal excretory function between recipients of a kidney from sympathectomized donors and recipients of a kidney from hydralazine-treated donors (Table 2). As a functional measure of glomerular damage, 24-h urinary protein excretion was measured in both groups. During both low- and high-NaCl diets, urinary protein excretion tended to be lower ($P < 0.053$) in recipients of a kidney from sympathectomized donors than in recipients of a graft from hydralazine-treated donors (Table 2). Graft weight was similar in both groups by the end of the experiments (Table 2). Histological examination of kidney grafts revealed a similar degree of tubular lesions, some interstitial infiltrations, and occasional glomerular lesions in either group and a trend toward more severe arteriosclerosis ($P < 0.06$) in recipients of grafts from hydralazine-treated donors (Fig. 5).

Effect of generalized reduction in sympathetic tone on hypertensive actions of SHR kidneys. Before transplantation of an SHR kidney, SAP and DAP were significantly less in neonatally sympathectomized F1H obtained from crossing SHR and WKY (111 ± 2 over 83 ± 2 mmHg) than in sham-treated F1H (139 ± 2 over 97 ± 2 mmHg) ($P < 0.001$). Heart rate was significantly higher (not shown) than in sham-treated F1H. Six weeks after renal transplantation, arterial pressure was elevated in both sympathectomized (135 ± 3 over 101 ± 2 mmHg) and sham-sympathectomized animals (183 ± 6 over 123 ± 4 mmHg) compared with pretransplantation values ($P < 0.001$). The arterial pressure rise was significantly more pronounced in sham-sympathectomized recipients. Data on MAP are summarized in Fig. 6. Heart rate and MAP variability were significantly higher in sympathectomized animals than in controls as evidenced by the standard deviation of the 30-min averages of the respective signals (Table 3). Pulse pressure was significantly less in sympathectomized recipients compared with sham-treated controls. Plasma catecholamine concentrations were significantly less in sympathectomized recipients of an SHR kidney than in sham-treated controls (Table 3).

Plasma creatinine and urea concentrations were 65.0 ± 3.3 μmol/l and 11.0 ± 0.5 mmol/l in sympathectomized recipients of an SHR kidney vs. 71.0 ± 4.8 μmol/l and 11.1 ± 0.5 mmol/l in sham-sympathectomized controls. Data did not differ significantly between both groups. Histological examination occasionally revealed some tubular dilations and mononuclear cells infiltrating the interstitium without differences between groups.

DISCUSSION

In the present study, we investigated the role of neonatal sympathetic tone on the ability of an SHR kidney to maintain arterial hypertension and the in-
fluence of a generalized reduction in sympathetic tone on the hypertensive action of a kidney graft obtained from young adult untreated SHR. We found that arterial pressure in SHR recipients of a kidney from neonatally sympathectomized SHR was less than in controls with a kidney from hydralazine-treated SHR, several weeks after renal transplantation. Furthermore, the arterial pressure rise after transplantation of an SHR kidney was less in neonatally sympathectomized F1H than in sham-treated F1H.

We showed previously that arterial pressure can be normalized in SHR when they are transplanted with a

Table 2. Plasma creatinine and urea concentrations, urinary protein excretion, body weight, and graft weight in recipients of a kidney from neonatally sympathectomized donors and recipients of a kidney from hydralazine-treated donors (n = 10 per group)

<table>
<thead>
<tr>
<th></th>
<th>Recipients of a Kidney from Sympathectomized Donors</th>
<th>Recipients of a Kidney from Hydralazine-Treated Donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma creatinine, µmol/l</td>
<td>60.0 ± 2.2</td>
<td>59.7 ± 3.5</td>
</tr>
<tr>
<td>Plasma urea, mmol/l</td>
<td>11.1 ± 1.3</td>
<td>10.6 ± 1.3</td>
</tr>
<tr>
<td>Proteinuria, low-NaCl diet, mg/days</td>
<td>24.0 ± 2.1</td>
<td>26.1 ± 1.6</td>
</tr>
<tr>
<td>Proteinuria, high-NaCl diet, mg/days</td>
<td>29.3 ± 2.1*</td>
<td>40.1 ± 4.1*</td>
</tr>
<tr>
<td>Graft weight, g</td>
<td>1.48 ± 0.05</td>
<td>1.43 ± 0.05</td>
</tr>
<tr>
<td>Final body weight, g</td>
<td>307 ± 8</td>
<td>318 ± 8</td>
</tr>
<tr>
<td>Graft weight/body weight, g/100 g</td>
<td>0.49 ± 0.07</td>
<td>0.45 ± 0.06</td>
</tr>
</tbody>
</table>

Values are means ± SE. Data did not differ significantly between groups; *P < 0.01 vs. respective data on low-NaCl diet.

Fig. 4. Top: renal function curves in SHR transplanted with a kidney from a hydralazine-treated donor (●, n = 10) and in SHR transplanted with a kidney from a neonatally sympathectomized donor (●, n = 10). Arterial pressure was significantly higher in recipients of a kidney from hydralazine-treated donors when fed a high-salt diet (1.8% NaCl) (*P < 0.05). Bottom: mean values of the inverse slopes of renal function curves from recipients of a kidney from hydralazine-treated donors (gray bar) and from recipients of a kidney from sympathectomized donors (black bar) (*P < 0.05).

Fig. 5. A: photomicrograph of a kidney graft from a neonatally sympathectomized donor (hematoxylin eosine). B: photomicrograph of a kidney graft from a hydralazine-treated donor (hematoxylin eosine). C: arterial vessel of a kidney graft from a hydralazine-treated donor. There was a trend toward more severe vascular lesions in kidney grafts from hydralazine-treated donors compared with kidney grafts from sympathectomized donors such as arteriosclerosis, neointima formation (arrow), and endothelial necrosis.
In contrast to other groups who reported arterial pressure normalization in SHR after neonatal sympathectomy (10, 23, 24), we did not observe a complete arterial pressure normalization in our rats. This difference may be explained, at least in part, by different arterial pressure recording conditions such as general anesthesia (10) or tail-cuff measurements (23, 24) and by slight differences in treatment regimens to induce neonatal sympathectomy (23, 24).

During the 30-day observation period, which started 2 wk after transplantation, MAP remained fairly constant ~140–145 mmHg in recipients of a kidney from sympathectomized donors. In contrast, MAP rose significantly and final MAP values were ~20 mmHg higher in recipients of a kidney from hydralazine-treated donors. This indicates that neonatal interruption of sympathetic tone directed to the kidney induces chronic changes in renal function resulting in an arterial pressure reduction in SHR even when extrarenal sympathetic tone is restored.

It is unlikely that this arterial pressure reduction was due to reduced neonatal arterial pressure in guanethidine-treated donors compared with hydralazine-treated animals that were given isotonic saline injections before weaning. We showed previously (28) that F1H recipients of a kidney graft from hydralazine-treated SHR developed posttransplantation hypertension even when the antihypertensive treatment of the donors was started in utero and continued during the postnatal period until the removal of the graft for renal transplantation at the age of 8 wk. Arterial pressure in these animals was not significantly different from that in F1H transplanted with a kidney from untreated SHR donors (28).

A prominent feature of SHR kidneys that may be involved in the development of hypertension is increased RVR (1, 30). Increased RVR can be detected with arterial pressure rise in SHR compared with WKY (32). SHR renal vasculature shows increased media thickness and an elevated number of smooth muscle cell layers compared with WKY even when hypertension development is largely prevented by chronic hydralazine treatment (31). Afferent arteriolar diameter has been shown to negatively correlate with arterial pressure in an F2 population derived from SHR and WKY (26). Intracellular Ca^{2+} mobilization in response to norepinephrine administration in

Table 3. HR, HR variability, MAP variability, pulse pressure, plasma NE, and EPI concentrations in sympathectomized and sham-treated F1H with an SHR kidney graft 6 wk after transplantation

<table>
<thead>
<tr>
<th></th>
<th>Sympathectomized F1H with SHR Kidney Graft (n = 10)</th>
<th>Sham-Treated F1H with SHR Kidney Graft (n = 8)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>436 ± 14</td>
<td>373 ± 8</td>
<td>0.001</td>
</tr>
<tr>
<td>HR SD, beats/min</td>
<td>46 ± 4</td>
<td>30 ± 2</td>
<td>0.001</td>
</tr>
<tr>
<td>MAP SD, mmHg</td>
<td>13 ± 1</td>
<td>10 ± 1</td>
<td>0.01</td>
</tr>
<tr>
<td>Pulse pressure, mmHg</td>
<td>35 ± 2</td>
<td>60 ± 3</td>
<td>0.001</td>
</tr>
<tr>
<td>Plasma NE, nmol/l</td>
<td>0.67 ± 0.14</td>
<td>1.88 ± 0.19</td>
<td>0.001</td>
</tr>
<tr>
<td>Plasma EPI, nmol/l</td>
<td>0.19 ± 0.02</td>
<td>0.33 ± 0.33</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are means ± SE. Standard deviations (SD) of individual heart rate (HR) and MAP recordings were used as a time-domain measure of HR and MAP variability. HR and MAP were recorded for 30 min in each animal. NE, norepinephrine; EPI, epinephrine; F1H, F1-hybrids.

Sympathetic Activity and Hypertension

Kidney from normotensive donors (14), indicating that the kidney is critical for the maintenance of hypertension in these animals. On the other hand, transplantation of an SHR kidney results in the development of hypertension in the recipients, which does not depend on sympathetic reinnervation of the SHR kidney graft (15). Renal denervation performed in 7-wk-old SHR delays hypertension development by ~4 wk (33), whereas renal denervation in young uninephrectomized SHR (15) and in adult SHR (33) has no effect on arterial pressure. Given the critical role of the kidney for the maintenance of hypertension in SHR and the long-lasting arterial pressure reduction in SHR after neonatal sympathectomy, we investigated the role of neonatal sympathetic innervation of the kidney for the maintenance of hypertension in these animals by means of a transplantation experiment. To control for unspecific effects of chronic arterial pressure reduction in sympathectomized donors, kidney donors of the control group were treated with hydralazine to lower arterial pressure by a similar magnitude.

In contrast to other groups who reported arterial pressure normalization in SHR after neonatal sympathectomy (10, 23, 24), we did not observe a complete arterial pressure normalization in our rats. This difference may be explained, at least in part, by different arterial pressure recording conditions such as general anesthesia (10) or tail-cuff measurements (23, 24) and by slight differences in treatment regimens to induce neonatal sympathectomy (23, 24).

During the 30-day observation period, which started 2 wk after transplantation, MAP remained fairly constant ~140–145 mmHg in recipients of a kidney from sympathectomized donors. In contrast, MAP rose significantly and final MAP values were ~20 mmHg higher in recipients of a kidney from hydralazine-treated donors. This indicates that neonatal interruption of sympathetic tone directed to the kidney induces chronic changes in renal function resulting in an arterial pressure reduction in SHR even when extrarenal sympathetic tone is restored.

It is unlikely that this arterial pressure reduction was due to reduced neonatal arterial pressure in guanethidine-treated donors compared with hydralazine-treated animals that were given isotonic saline injections before weaning. We showed previously (28) that F1H recipients of a kidney graft from hydralazine-treated SHR developed posttransplantation hypertension even when the antihypertensive treatment of the donors was started in utero and continued during the postnatal period until the removal of the graft for renal transplantation at the age of 8 wk. Arterial pressure in these animals was not significantly different from that in F1H transplanted with a kidney from untreated SHR donors (28).

A prominent feature of SHR kidneys that may be involved in the development of hypertension is increased RVR (1, 30). Increased RVR can be detected with arterial pressure rise in SHR compared with WKY (32). SHR renal vasculature shows increased media thickness and an elevated number of smooth muscle cell layers compared with WKY even when hypertension development is largely prevented by chronic hydralazine treatment (31). Afferent arteriolar diameter has been shown to negatively correlate with arterial pressure in an F2 population derived from SHR and WKY (26). Intracellular Ca^{2+} mobilization in response to norepinephrine administration in

Fig. 6. MAP in sympathectomized F1-hybrids (F1H; ○, n = 10) and sham-treated F1H (○, n = 8) before transplantation with an SHR kidney and 6 wk after renal transplantation. *Significant difference between groups (P < 0.001). †Significant interaction between the factor treatment (sympathectomy, sham treatment) and time after transplantation of an SHR kidney (P < 0.05).
SHR renal afferent arterioles is less sensitive to blockade of IP₃ receptors than in WKY afferent arterioles (30). Sympathetic renal innervation develops faster during the postnatal period in SHR than in WKY rats (12), and renal sympathetic nerve activity is elevated in SHR (8, 20, 25). Catecholamines are potent vasoconstrictors, stimulate proliferation and growth of vascular smooth muscle cells (5, 34), and may be critically involved in the development of increased RVR in SHR (8).

Neonatal sympathectomy results in a reduction in hindlimb vascular resistance (10, 23) and a reduction in mesenteric wall/lumen ratio (24) in SHR. In the present study, we extended these findings (10, 23, 24) to the renal vasculature by showing that RVR was reduced in neonatally sympathectomized SHR compared with sham-treated controls. Although the present data do not provide direct evidence, reduced RVR of kidney grafts from sympathectomized donors compared with grafts from hydralazine-treated donors may have contributed to the lower arterial pressure values observed in recipients of a kidney from donors subjected to neonatal sympathectomy.

Sodium sensitivity of arterial pressure was less in recipients of a kidney from sympathectomized donors than in recipients of a kidney from hydralazine-treated donors. The intercepts of the renal function curves with the MAP axis were not significantly different between both transplanted groups, indicating a similar nonsodium-sensitive component of arterial pressure (21). We could not detect any group differences in sodium intake and renal sodium excretion during the metabolism experiment. A limitation of these observations may be that transient group differences in renal sodium balance of only a few hours in response to changes in sodium intake may have remained undetected due to sampling periods of 24 h.

Although MAP in both recipients of a kidney from sympathectomized and hydralazine-treated donors remained in the hypertensive range, our data on the relation between MAP and natriuresis resemble data obtained in uninephrectomized animals showing a decreased slope of the pressure-natriuresis curve in 6- to 9-wk-old and in adult SHR compared with WKY with major neurohormonal factors clamped to identical levels (29).

The mechanisms leading to different renal function curves in recipients of a kidney from sympathectomized donors and in recipients of a kidney from hydralazine-treated donors remain to be investigated. These may involve reduced RVR and an enhanced perfusion pressure-dependent increase in renal medullary blood flow, which contributes to pressure natriuresis and arterial pressure regulation (7). Renal medullary blood flow depends on the balance between vasoconstricting and vasodilating mechanisms (7, 9, 35). This balance appears to be shifted toward vasoconstricting mechanisms in SHR compared with normotensive rats (7, 9). It has been shown that angiotensin I-converting enzyme inhibitor treatment from postnatal weeks 4-14 chronically improves the function of the SHR renal medullary NO system, which may, in turn, enhance pressure natriuresis in these animals (9). Furthermore, neonatal sympathectomy may have improved the regulation of renin secretion, intrarenal paracrine mechanisms involved in sodium handling, and/or components of epithelial sodium transport in a way that arterial pressure in recipients of a kidney from sympathectomized donors was less sodium sensitive.

Kidney weight and kidney weight-to-body weight ratio were similar in hydralazine-treated and in sympathectomized rats. Graft weights did not differ significantly between SHR recipients of a kidney from hydralazine-treated or sympathectomized SHR donors by the end of the experiments. These data indicate that major differences in renal mass did not contribute to differential arterial pressure and sodium sensitivity in the respective recipient groups. It remains to be investigated if neonatal sympathectomy caused more subtle changes in SHR nephron morphology compared with hydralazine-treated controls (3).

We showed previously that hypertension induced by an SHR kidney graft does not depend on graft reinnervation (15) or elevated extrarenal sympathetic tone (13). However, these results (13, 15) do not indicate that sympathetic activity is not involved in setting the arterial pressure level in recipients of an SHR kidney graft. Moreover, we and others recently showed that arterial pressure in recipients of an SHR kidney with both native kidneys removed depends on the amount of SHR genome carried by the recipient (6, 14), suggesting that extrarenal mechanisms contribute to long-term arterial pressure in these animals. A likely candidate for differentially acting extrarenal pressor systems is the sympathetic nervous system. Therefore, we investigated the contribution of extrarenal sympathetic tone to hypertension development after transplantation of an SHR kidney obtained from untreated donors.

Because of the difficulties to reliably quantify chronic levels of sympathetic activity in small experimental animals by means of direct recordings (2, 4), we chose to chronically lower sympathetic tone in one recipient group by neonatal guanethidine treatment and removal of adrenal medullary tissue. Lower plasma catecholamine concentrations as measured in the sympathectomized recipient group compared with controls indicate that a generalized reduction in sympathetic tone was achieved. Unfortunately, this parameter does not provide a quantitative measure for the degree of reduction in sympathetic tone under varying physiological conditions because both release and reuptake of catecholamines are impaired after guanethidine-induced lesions of sympathetic nerve terminals (19). Nevertheless, the finding of reduced plasma catecholamine levels is important for the present study, because plasma is the main source of sympathetic transmitters for kidney grafts. Further evidence for a successful reduction of sympathetic tone is provided by the observed elevations in heart rate, heart rate variability, and arterial pressure variability as well as the reduced arterial pressure.
After transplantation of an SHR kidney, arterial pressure rose in both sympathectomized and sham-treated recipients. However, the extent of the arterial pressure rise and final arterial pressure values were significantly less in sympathectomized than in sham-treated recipients 6 wk after transplantation. The kidney-fluid system is regarded as central for long-term arterial pressure control (16). The present data suggest that this system can be reset to a lower arterial pressure level by a chronic generalized reduction of sympathetic tone. Furthermore, these data provide experimental evidence for the hypothesis that chronic nonadapting changes in sympathetic activity may have a major impact on long-term arterial pressure regulation (4, 16). The mechanisms underlying the arterial pressure difference between sympathectomized and sham-treated recipients of an SHR kidney remain currently unknown. It is conceivable that different amounts of catecholamines reaching the kidney via the blood play a major role. It has been shown that norepinephrine infused into the renal artery in doses that have no pressor action when delivered into the systemic circulation induces hypertension in normotensive rats (22).

With regard to sodium chloride-induced hypertension, it has been hypothesized that the failure of an organism to suppress sympathetic activity when challenged with a high-NaCl diet rather than the level of basal sympathetic activity may contribute to hypertension (4). This appears not to be the case in renal posttransplantation hypertension, which is associated with increased renal sodium retention (11). Data on heart rate and arterial pressure variability in sympathectomized F1H of the present study suggest that the range by which efferent sympathetic activity can be modified is reduced to a great extent, but the increase of arterial pressure in response to an SHR kidney graft is less than in F1H with an intact sympathetic nervous system.

In conclusion, we showed that neonatal sympathetic innervation of SHR kidneys contributes to the development of chronic changes in renal function such as increased RVR and compromised renal sodium excretion, which may be involved in the development of hypertension. Furthermore, there is a strong interaction between renal and extrarenal factors such as sympathetic activity in setting long-term arterial pressure.

Perspectives

The kidney has long been regarded as the dominant system of long-term arterial pressure control. This is supported by experiments showing arterial pressure normalization in genetically hypertensive rats by a kidney graft from normotensive animals. However, with regard to SHR, primary hypertension evidence is accumulating that the quantitative contribution of the kidney to the chronic arterial pressure level can be greatly modified (i.e., reduced) by extrarenal factors. Furthermore, the absolute arterial pressure level of rats with an SHR kidney appears to depend on both renal and extrarenal factors. In other words, extrarenal and renal factors may interact to determine chronic arterial pressure. It remains to be discriminated to what extent structural and physical factors such as peripheral vascular resistance and neurohormonal factors contribute to the interaction between renal and extrarenal mechanisms in long-term arterial pressure control. With regard to the influence of the sympathetic nervous system on the development of SHR kidney function, future research should investigate in greater detail what aspects of renal vascular and epithelial function are chronically modified by neonatal sympathectomy.

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