Role of the renal sympathetic nerves in renal sodium/potassium handling and renal damage in spontaneously hypertensive rats

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Abstract. Renal sympathetic nerve activity has an important role in renal disease-associated hypertension and in the modulation of fluid homeostasis. In the present study, changes in renal function and renal sodium/potassium handling were investigated in groups of 12-week-old male, spontaneously hypertensive rats with renal denervation (RDNX group) or sham denervation (sham group). The RDNX group excreted significantly more sodium than the sham group during the 2-week observation period (P<0.05). Following bilateral renal denervation, the fractional lithium excretion was elevated in the RDNX group compared with the sham group, but no significant effect was observed of renal denervation on the fractional distal reabsorption rate of sodium or the fractional excretion of potassium. Furthermore, the glomerular injury score and the wall-to-lumen ratio of the interlobular artery were significantly lower in the RDNX group than in the sham group (P<0.05). In conclusion, the present study indicates an involvement of the renal sympathetic nerves in the regulation of renal tubular sodium reabsorption in spontaneously hypertensive rats and in the renal damage associated with hypertension.

Introduction

Hypertension causes renal injury and is a major factor inducing progressive organ damage in end-stage renal disease (1). Sympathetic nerve activity (SNA) is increased in patients with chronic kidney disease and has an important and distinct role in renal disease-associated hypertension (2,3). A long-standing hypothesis proposes that neurogenic hypertension results from alterations to renal function via the actions of renal nerves on renal vascular resistance, tubular sodium reabsorption and renin release (4,5). Catheter-based renal denervation (RD) has been introduced into clinical practice to selectively denervate efferent and afferent renal sympathetic fibers (6-8). Other than resulting in marked and sustained blood pressure reductions, RD has also been demonstrated to reduce renal resistive index, and the incidence of albuminuria without adversely affecting glomerular filtration rate or renal artery structure (9).

The kidneys are critical to the regulation of blood pressure via modulation of sodium and water excretion (10). One mechanism by which the kidneys are posited to maintain fluid homeostasis is using the renal sympathetic nerves (11). RD is widely reported to cause an increase in sodium, potassium and water excretion in several mammalian species (12-15). However, controversy remains regarding the immediate changes to renal excretory functions following RD (15,16). No appreciable differences were observed by Salman *et al* in the mean arterial pressure (MAP) and plasma sodium (PNa) between denervated and innervated SD rats (15,16).

In the present study, the changes to renal function and renal sodium/potassium handling were investigated in spontaneously hypertensive rats (SHR) subjected to RD, with the aim of functionally characterizing the sympathetic nerve control of the kidney. The current study addressed this aim using a correlation of renal sodium/potassium excretions and renal function by combining surgical and chemical procedures to achieve RD.

Materials and methods

Animals. All experimental procedures conformed to the Animal Ethics Committee guidelines of Guangxi Medical University (Nanning, China), and received committee approval. Twelve-week-old male SHR (n=16 animals) and age-matched Wistar Kyoto (WKY) rats (n=8 animals) were obtained from Vital River Laboratories Co., Ltd. (Beijing, China). The animals were housed in controlled environmental conditions consisting of a 12-h light/dark cycle and 22 ± 2˚C. All animals were fed *ad libitum* throughout the study with standard rat chow (Vital River Laboratories Co., Ltd.) for at least seven days before the study. Following an acclimation

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period of two weeks, SHR were randomly assigned to the renal denervated (RDNX; n=8) or sham (n=8) groups.

Surgical preparation of animal and experimental protocol. Rats were fasted overnight prior to the surgery. Briefly, anesthesia was induced using pentobarbital sodium at a dose of 50 mg/kg (intraperitoneally; Sigma-Aldrich, St. Louis, MO, USA), and the kidneys were exposed through midline abdominal incisions and surgically denervated with the aid of a stereomicroscope (Shanghai Third Optical Instrument Factory, Shanghai, China). Denervation was accomplished by incising all visible nerves along the renal artery, and the renal vessels were surrounded with cotton swabs previously soaked in 10% (v/v) phenol solution (Shanghai Biological Engineering Co., Ltd., Shanghai, China) for 2 min. Sham RD (sham group) treatment entailed identical anesthetic and surgical procedures, but the renal nerves were left intact. After two weeks, tail arterial pressure was estimated by the tail-cuff method (17), and urine was collected over 24 h. Under anesthesia with 200 mg/kg ketamine (intramuscular injection; Jiang Su Heng Rui Medicine Co., Ltd., Suzhou, China), blood was drawn via abdominal aortic puncture, and the kidneys were removed.

Serum and urine lithium concentrations were measured with an AA-7000 atomic absorption spectrometer (Shimadzu, Kyoto, Japan), while serum and urine sodium, potassium, protein and creatinine concentrations were determined by spectrophotometer (Roche Diagnostics, Basel, Switzerland). To confirm that the chemical RD was achieved in the rats, renal tissue and serum noradrenaline (NE) content was analyzed in each experimental group using high-performance liquid chromatography (HPLC) with electrochemical detection (ECD) (LC-20A; Shimadzu).

Calculations. Urine flow rate (UFR) was calculated by the following formula: UFR (μl/min/kg) = UV/T x BW, in which UV (μl) is the urine volume, T (min) is the time and BW (kg) is the body weight of the rat (16).

The clearances were calculated using the usual formula: $Cx = Ux \times UFR/Sx$, in which Cx is the clearance of substance 'x', Ux is the urine concentration of 'x' and Sx is the serum concentration of 'x'. Glomerular filtration rate (Ccr) was considered to indicate the clearance of creatinine. The fractional excretion of 'x' (FEx) was calculated as: Cx/Ccr. The fractional distal reabsorption rate of sodium was calculated by the following formula: $FDR_{Na} = [(FE_{Li}-FE_{Na})/FE_{Li}] \times 100$. FE_{Li} and FDR_{Na} were calculated as markers of proximal and distal sodium handling, respectively (18). $FE_{Li} = U_{Li} \times Scr/S_{Li} \times U_{cr}$. Serum and urine sodium, potassium, protein and creatinine concentrations were determined using a spectrophotometer (Roche Diagnostics) and lithium concentration by inductively coupled plasma mass spectrometry (7500CE; Agilent Technologies, Inc., Santa Clara, CA, USA).

Histological examination. Kidney tissues (coronal slices) were fixed with 10% paraformaldehyde and embedded in paraffin (Shanghai Biological Engineering Co., Ltd.). Coronal sections of the kidney (4-μm-thick) were stained with periodic acid-Schiff and Masson-trichrome stains (Fuzhou Maixin Biotech, Co., Ltd., Fuzhou, China), and examined blind using a DP72 brightfield microscope (Olympus Corporation,

Tokyo, Japan) to assess glomerular and arterial morphology. Histological scores were assessed using Image-Pro Plus version 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA). Glomerular injury score was calculated as previously described (19,20). At least 50 glomeruli were randomly selected in each rat and the mean glomerular injury score was calculated. The severity of tubulointerstitial injury was evaluated by the interstitial fibrosis (IF) score, as described previously (19,20). The percentage of interstitial fibrotic areas per cortical field (magnification, x100) was calculated, and the mean percentage from 10 randomly selected fields of view was determined as the IF score for each rat. The medial thickness-to-lumen ratio was calculated as described previously (21). For this, 5 regions of the interlobular artery from each rat were evaluated, and the average ratio was calculated.

Statistical analysis. Values are presented as mean ± standard error. Statistical analysis was performed using SPSS 16.0 software (SPSS, Inc., Chicago, IL, USA). Comparisons between multiple groups were evaluated using one-way analysis of variance followed by the Dunnett test. P<0.05 was considered to represent a statistically significant difference.

Results

General observations. The body weights of the rats were similar in all three groups (RDNX group, 231±15 g; sham group, 238±13 g; and WKY group, 229±16 g). RD was confirmed by assessment of renal tissue and serum NE content (Table I). As Table I shows that serum NE content was significantly lower in RDNX compared with the sham group (P<0.05), and did not differ between the RDNX and WKY groups. The kidney NE content was significantly lower in the RDNX group compared with the sham group (P<0.05), and did not differ between the RDNX and WKY groups. No significant differences in serum sodium, potassium or creatinine, and serum or urine protein concentrations were observed among the three groups. There was no observably significant difference in Ccr among the three groups. The kidney weight/body weight ratio of the rats was not affected by bilateral renal denervation (RDNX group, 3.515±0.14 g/kg; sham group, 3.64±0.29 g/kg; and WKY group, 3.5ww6±0.23 g/kg).

Effects of RD on renal sodium/potassium excretions. FE_K was lower in the RDNX group compared with the sham group, but no significant difference in FE_K was found among the three groups (Table I). RD generated a significantly (P<0.05) higher sodium clearance (C_{Na}) compared with the sham group (Fig. 1). Sham and WKY groups showed significant differences for FE_K and C_{Na}. Furthermore, the FELi was significantly (P<0.05) higher in the RDNX group compared with the sham group. FE_K, C_{Na} and FE_{Li} exhibited no significant differences between the RDNX and WKY groups. In contrast with these observations, RD did not significantly alter FDR_{Na} (P>0.05) in SHRs compared with the sham group.

Effects of RD on renal histopathology. Less glomerular morphological change, assessed by the glomerular injury score, was noted in the RDNX group compared with the sham group (Fig. 2). The glomerular injury score was lower in the

Table I. Parameters of renal function and sodium handling in rats.

Parameter	RDNX	Sham	WKY	P-value
Kw/Bw, g/kg	3.515±0.14	3.64±0.29	3.56±0.23	0.31
MAP, mmHg	96±7[a]	131±10	89±8[a]	<0.05
S-NE, ng/ml	14.02±2.37[a]	23.04±8.77	13.41±3.95[a]	<0.05
K-NE, ng/mg	0.95±0.21[a]	1.35±0.18	1.01±0.24[a]	<0.05
S-Na, mmol/l	142.78±1.09	142.12±1.36	141.43±1.22	0.67
S-K, mmol/l	4.56±0.29	4.88±0.32	4.51±0.24	0.17
S-Cr, mmol/l	30.78±3.96	32.88±4.09	34.00±3.21	0.26
S-Pro, g/l	55.16±7.68	57.19±5.31	60.57±8.60	0.23
U-Pro, mg/24 h	2.35±0.42	2.35±0.74	2.07±0.47	0.54
FE_K, %	47.04±4.80	52.77±4.78	45.75±6.41	0.19
FE_{Li}, %	18.13±2.21[a]	15.24±1.78	17.35±2.17[a]	0.03
C_{Na}, μl/min	5.39±1.83[a]	3.63±1.27	5.59±1.97[a]	0.04
FDR_{Na}, %	94.55±9.33	94.21±7.01	93.97±10.65	0.08
Ccr, ml/min·kg	0.48±0.13	0.45±0.15	0.52±0.14	0.60

Values are reported as the mean ± standard error (n=8). Kw, kidney weight; Bw, body weight; MAP, mean arterial pressure; S-NE, serum noradrenaline content; K-NE, kidney noradrenaline content; S-Na, serum sodium; S-K, serum potassium; S-Cr, serum creatinine; S-Pro, serum total protein; U-Pro, urinary protein; FE_K, fractional excretion of potassium into urine; FE_{Li}, fractional excretion of lithium into urine; C_{Na}, sodium clearance; FDR_{Na}, fractional distal reabsorption rate of sodium; Ccr, creatinine clearance. [a]P<0.05 vs. sham group.
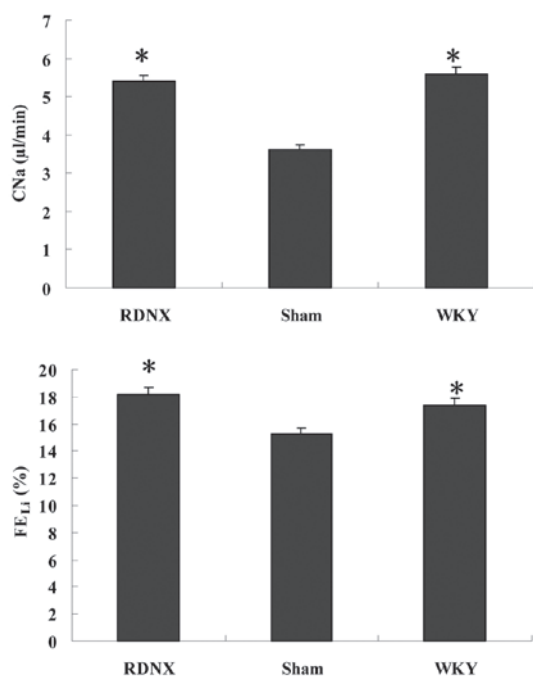

Figure 1. C_{Na} and FE_{Li} in rats. C_{Na}, sodium clearance; FE_{Li}, fractional lithium excretion. Data are reported as the mean ± standard error of the mean. *P<0.05 vs. sham group.

RDNX group than in the sham group (2.5±0.1 vs. 0.7±0.2, respectively; P<0.05). To assess changes to the inter-lobular artery, a parameter of hypertension-related vascular damage, the wall-to-lumen ratio was examined. The medial thickness-to-lumen diameter ratio was higher in the sham group than in the WKY group, indicating that this had been caused by sham treatment (Fig. 3). RD significantly decreased

the ratio to a similar level as that of the WKY group. The severity of tubulointerstitial injury was evaluated by IF score, revealing no significant difference in IF score among the three groups (Fig. 4).

Discussion

Renal sympathetic nerves utilize NE as a neurotransmitter, which affects the renal arterioles, juxtaglomerular granular cells and tubules of the kidney (22). Using a rat model of genetic hypertension, several manifestations of renal sodium excretion, glomerular and arterial morphological structure was reported in the present study to be substantially alleviated when RD is performed by chemical sympathectomy.

In the current study, SHRs were selected due to previous evidence of increased renal sympathetic discharge in this hypertension model (23). The kidney has an important role in the regulation of blood pressure via modulation of sodium and water excretion (10). A previous study of SHRs suggested that an impaired pressure-diuresis relationship exists in these animals, such that greater perfusion pressures are required to achieve the same level of diuresis when compared with WKY rats (24). Furthermore, a previous study using isolated perfused kidneys from SHRs revealed an intrinsic renal abnormality in sodium excretion that may contribute to the maintenance of hypertension in SHR (25). Renal sympathetic nerves and circulating catecholamine are involved in the regulation of sodium and water excretion in the kidney (26). In the present study, a significant change to sodium excretion was observed following RD. Higher C_{Na} was observed in the denervated SHRs compared with rats with intact renal nerves. The observed natriuresis following RD has been reported in several studies on acute and chronic RD of different experimental hypertensive rats (21,27,28). Together, these
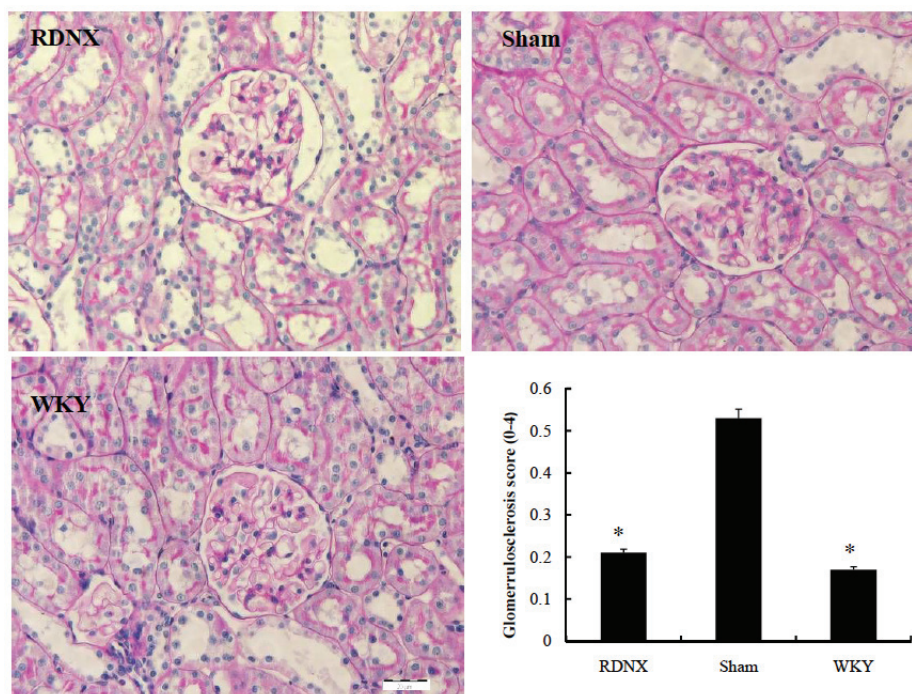
Figure 2. Changes in the glomeruli following renal denervation, detected using periodic acid-Schiff staining. Glomerular injury caused by mesangial expansion was graded from 0 to 4. At least 50 glomeruli, selected at random, were assessed from each rat and the mean scores were calculated and compared. *P<0.05 vs. sham group.
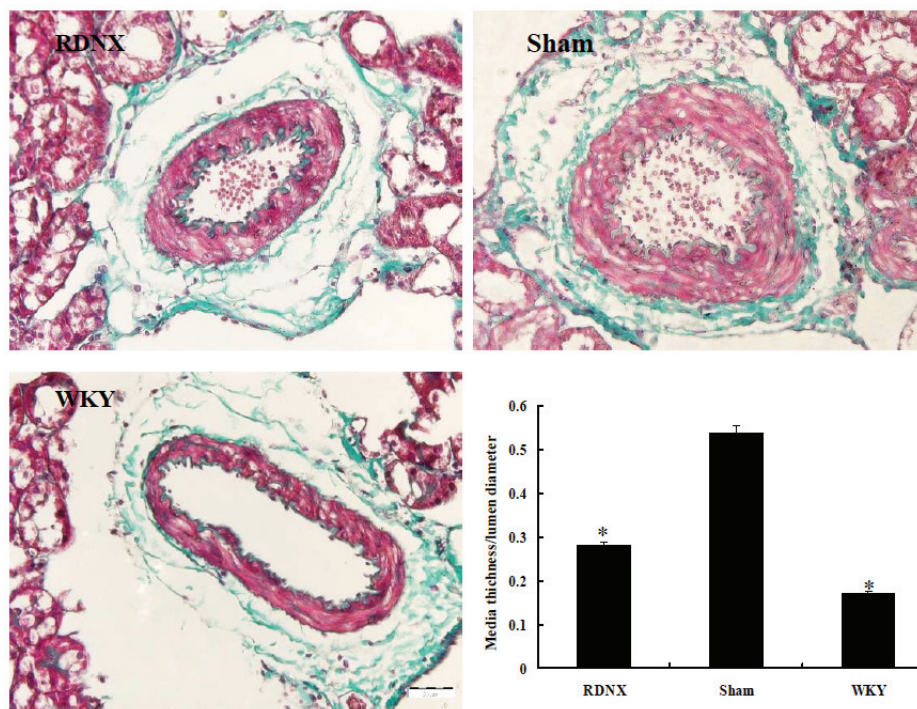

Figure 3. Changes in the interlobular artery following renal denervation, observed using Masson staining. The interlobular artery was identified as a single muscular artery within the inner cortex, sometimes appearing adjacent to the glomerulus. Five arteries were examined from each rat, from which the medial thickness-to-lumen ratio was averaged for each rat and group means were calculated and compared. *P<0.05 vs. sham group.

and previous results implicated renal sympathetic activity in sodium regulation (16).

It has previously been revealed that NE released from renal sympathetic nerve endings acts on the basolateral membranes of epithelial cells to stimulate tubular sodium and water reabsorption at the proximal tubule, thick ascending limb of the loop of Henle and the distal nephron (29). Electric stimulation of the renal nerves in acute experiments has been demonstrated to enhance sodium reabsorption, particularly in the proximal convoluted tubule. Low-frequency renal nerve
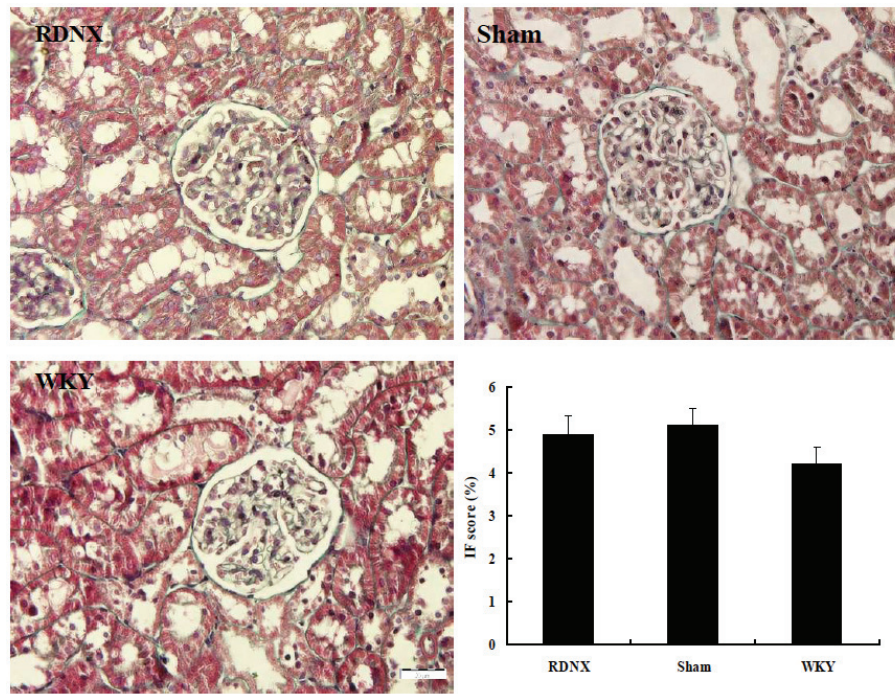
Figure 4. Histology of the tubulointerstitium, detected by Masson staining. The severity of tubulointerstitial injury was evaluated by IF score. The percentage of interstitial fibrotic areas per cortical field (magnification, x400) was calculated, and the mean percentage in 10 randomly selected fields was determined as the IF score for each rat. No significant differences were observed in IF score among the three groups.

stimulation directly affects proximal tubular sodium reabsorption and rennin release in the absence of changes to renal hemodynamics (30). Furthermore, several previous studies indicated that RD results in an increased urine flow rate that is attributed to a decreased absolute and fractional reabsorption of sodium in the proximal convoluted tubule (31,32). Conversely, several previous studies have reported that bilateral RD in 3- to 8-week-old SHRs delays the development of hypertension, associated with reduced sodium reabsorption by the proximal tubule, the loop of Henle and the distal convolution (33-35). In the present study, following bilateral RD, FE_{Li} was elevated when compared with the sham-operated group, consistent with the withdrawal of sympathetic stimulation. While the results of the present study indicated no significant effect of RD on FDR_{Na}, it may be that RD results in decreased sodium reabsorption by the proximal convoluted tubule, but not the distal convoluted tubule.

The renal mechanisms of potassium excretion have varied upon the application of a diversity of techniques including renal clearance, micropuncture, microperfusion and electrophysiological studies (15). However, a paucity of information exists on the role of the adrenergic mechanisms in the regulation of renal potassium reabsorption and secretion. It is established that potassium freely diffuses in the renal corpuscular membrane, and the majority of its filtered load is reabsorbed by proximal tubular epithelial cells. However, potassium excretion in the urine depends on controlled secretion in the distal nephron (36). Salman et al (15) demonstrated that RD in Sprague Dawley rats caused a significantly higher renal potassium excretion in absolute terms and as a fraction of the filtered load. However, the present study did not report significant changes to plasma potassium levels and urinary potassium excretion, suggesting that decreased renal sympathetic nerve

activity of SHR has a more direct tubule natriuretic effect in the proximal segment than at the sodium-potassium exchange site.

Hypertension is an established consequence of chronic renal disease, and is often observed in patients with focal segmental glomerulosclerosis and with membranoproliferative glomerulonephritis (37). Furthermore, several lines of evidence suggest that sympathetic overactivity, through functional and morphological alterations to renal physiology and structure, may contribute to kidney injury and chronic kidney disease progression (38). In the present study, renal sympathetic denervation aided amelioration of glomerular sclerosis in SHR. To assess changes to the small arteries, a parameter of hypertension-related vascular damage, the wall-to-lumen ratio was examined. The current data indicated that RD not only reverses glomerular sclerosis, but also greatly enlarges the lumen size of the interlobular artery in these genetically hypertensive rats. There is much experimental evidence and clinical data to indicate that drugs that reduce SNA have a renoprotective effect. Moxonidine, which decreases sympathetic nerve activity, was revealed to have renoprotective effects in patients, in addition to in experimental rats with renal failure (39-41). Furthermore, catheter-based RD selectively targets efferent and afferent renal nerves and functionally denervates the kidney, reducing blood pressure in clinical trials (38), and provide renoprotection in diabetic and Dahl salt-sensitive rats by ameliorating the effects of excessive renal sympathetic signals (3,42). Together, these observations confirm that RD considerably improves glomerular sclerosis and hypertension-associated renal vascular damage, which indicates a role of the overactive sympathetic nervous system in this pathophysiological state.

In conclusion, the present data demonstrate that renal nerves are significantly involved in the regulation of renal tubular

sodium reabsorption in SHR. Furthermore, if the kidney is prevented from sympathetic nerve stimulation, structural changes due to early stage hypertensive nephropathy, namely glomerular sclerosis and vascular damage, are abolished.

Acknowledgements

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