Renal Structural Properties in Prehypertensive Dahl Salt-Sensitive Rats

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Abstract—In 10- to 12-week-old Dahl salt-sensitive (DS) and salt-resistant (DR) rats fed a 0.3% salt diet (n=10 in each group), flow-pressure and pressure–glomerular filtration rate (F-P and P-GFR, respectively) relationships were established for maximally vasodilated perfused kidneys. From these relationships, 3 indices of vascular structural properties were estimated: slope of F-P (minimal renal vascular resistance reflecting overall luminal dimensions of preglomerular and postglomerular vasculature), slope of P-GFR (glomerular filtration capability against pressure), and threshold pressure for beginning filtration at P-GFR (preglomerular-to-postglomerular vascular resistance ratio). Thereafter, maximal renal vascular resistance was determined to assess wall-to-lumen ratios of the resistance vessels in half of each group. In the remainder, the kidneys were perfusion-fixed for histological analysis. Mean arterial pressure did not differ between the DS and DR rats. There were no significant differences in the slopes of F-P between the 2 groups. In contrast, the slope of P-GFR was significantly lower (33%) in DS rats than in DR rats, although the DS kidneys began filtering at a threshold pressure similar to that of the DR kidneys. Thus, in DS rats, there were no abnormalities in luminal dimensions at preglomerular and postglomerular vascular segments, but the kidney filtration capacity decreased at any given increase in pressure. Maximal vascular resistance was greater in DS than in DR rats, a finding compatible with the histological appearance, which showed vascular hypertrophy with little, if any, vascular narrowing in the interlobular arteries of DS rats. In conclusion, hypertrophic remodeling without vascular narrowing at preglomerular resistance vessels and structural defects in filtering at the glomeruli could occur in prehypertensive DS rats. (Hypertension. 2000;36:68-72.)

Key Words: renal artery ▪ rats, Dahl ▪ kidney ▪ remodeling ▪ hypertension, sodium-dependent

On the basis of intrarenal hemodynamic data in various hypertensive animal models, it is recognized that in models of salt-induced hypertension, including Dahl salt-sensitive (DS) rats and deoxycorticosterone-salt rats, intraglomerular pressure is elevated compared with models of spontaneous (ie, salt–salt-induced) hypertension, such as the spontaneously hypertensive rat(s) (SHR).1–6 Because intraglomerular hypertension plays an important role in the genesis of glomerular injuries,7 it might be responsible for the early onset and rapid progression of hypertensive renal damages that are found in association with salt-induced hypertension in animals and humans.8–13 Accordingly, from the viewpoint of cardiovascular protection, it is clinically important to elucidate the precise mechanisms causing intraglomerular hypertension in salt-induced hypertension.

In hypertension, it is well known that resistance vessels become thicker or encroach into the lumen (ie, vascular hypertrophy or vascular remodeling) in the kidneys as well as in other vascular beds.14,15 In such hypertrophic or remodeled resistance vessels, vasoconstriction could be potentiated by increased constrictive force or greater vascular narrowing in response to any given level of vasoconstrictor stimuli.14–16 Therefore, the vascular structural property is considered to be one of the important factors determining the intrarenal hemodynamics in hypertension. Actually, vascular remodeling, vascular hypertrophy, or both occur mainly in the preglomerular resistance vessels, leading to vascular luminal narrowing in the prehypertensive or early hypertensive stage seen in SHR.17–23 Thus, in the spontaneous form of hypertension, such as that seen in SHR, it is assumed that these structural changes would promote an increase in renal vascular resistance, especially in the preglomerular vasculature, and thereby serve to maintain normal glomerular pressure in the face of systemic hypertension.24 The intrarenal hemodynamics characterized by these structural properties is consistent with the hemodynamics obtained in SHR in vivo.25,26 Similarly, there are previous reports examining the renal vascular structural designs in salt-induced hypertensive models.27–30 However, these results of these reports were obtained mainly at the stage of established hypertension, which could be confounded by the nonspecific pressure-mediated structural effects, and did not represent the real structural properties specific for salt-induced hypertension.
Therefore, the purpose of the present study was to investigate the structural properties of renal resistance vessels at the prehypertensive stage of DS rats, one of the salt-induced hypertensive models, with Dahl-salt resistant (DR) rats used as controls. The renal structural properties were estimated by measuring their hemodynamic behavior and histological appearance in vitro, maximally vasodilated, perfused kidneys. To the best of our knowledge, the present study was the first to demonstrate the renal structural characteristics that might be involved in the genesis of glomerular hypertension in DS rats.

Methods

Animals and Procedures

Five-week-old male DS and DR rats that were obtained from the Mollegaard Breeding Center (Ejby, Denmark) were bred and supplied by Seiwa Experimental Animals Ltd (Fukuoika, Japan) (n=10 in each group). Standard laboratory rat chow containing 0.3% NaCl and tap water were supplied ad libitum. The rats were housed in a room maintained at constant temperature (23°C to 25°C) with a 12-hour light/dark cycle until the initiation of the experiments. When they were 10 to 12 weeks old, each rat was subjected to the experiments described below. The study design and experimental protocols were in accordance with our institutional guidelines for animal research.

The rats were anesthetized with pentobarbital sodium (60 mg/kg IP) and placed on a warm operating table. Once a surgical level of anesthesia had been established, tracheotomy and cannulation of the left jugular vein by a polyethylene catheter (PE-50) were performed, and then a continuous infusion of pentobarbital sodium in saline (5 mg/h) was begun via the left jugular vein. After blood pressure was measured via a catheter (PE-50) inserted into the tail artery, a midline abdominal incision was performed. The urine was obtained from the bladder for measurement of urinary protein and creatinine levels with the use of 2 commercial kits (Bio-Rad protein assay; creatinine assay kit, Boehringer-Mannheim). Thereafter, the intestines were removed, and the abdominal aorta was isolated 1 cm proximally and distally to the left renal artery. The left ureter was cannulated (PE-10, 6 cm) for collection of urine, and the mesenteric artery was cannulated (PE-50) for measurement of aortic pressure close to the left renal artery. This pressure was considered to be the renal arterial inflow pressure. All visible branches of the isolated aorta were ligated except for the left renal artery and mesenteric artery. After heparinization (3000 U/kg IV) and ligation of the abdominal aorta at the bifurcation of the iliac arteries, a PE-90 catheter connected to the perfusion setup was inserted retrogradely through the abdominal aorta to a position distal to the left kidney. The left ureter was cannulated (PE-50) inserted into the tail artery, a midline abdominal incision was performed. The urine was obtained from the bladder for measurement of urinary protein and creatinine levels with the use of 2 commercial kits (Bio-Rad protein assay; creatinine assay kit, Boehringer-Mannheim). Thereafter, the intestines were removed, and the abdominal aorta was isolated 1 cm proximally and distally to the left renal artery. The left ureter was cannulated (PE-10, 6 cm) for collection of urine, and the mesenteric artery was cannulated (PE-50) for measurement of aortic pressure close to the left renal artery. This pressure was considered to be the renal arterial inflow pressure. All visible branches of the isolated aorta were ligated except for the left renal artery and mesenteric artery.

After heparinization (3000 U/kg IV) and ligation of the abdominal aorta at the bifurcation of the iliac arteries, a PE-90 catheter connected to the perfusion setup was inserted retrogradely through the abdominal aorta to a position distal to the left kidney. The aorta was tied off just above the mesenteric artery to make a closed arterial circuit, including the left kidney, and the renal vein was cut. Then perfusion of the left kidney was begun with oxygenated (95% O\textsubscript{2} and 5% CO\textsubscript{2}) artificial perfusate by use of a peristaltic pump (Advantec Tokyo Kaisha Ltd), and the flow rate was initially maintained at 3.0 mL/min. The rats were killed by an overdose of pentobarbital, and the renal capsule was removed to minimize increments in tissue pressure during the kidney perfusion. The adrenal glands were also removed to eliminate the confounding effects of glucocorticoids or mineralocorticoids on the hemodynamic analysis. A needle (25 gauge, inner diameter 0.3 mm) was inserted into the midcortex for measurement of intrarenal tissue pressure. The perfusate consisted of a modified Tyrode’s solution containing 148 mmol/L Na\textsuperscript{+}, 4.3 mmol/L K\textsuperscript{+}, 133 mmol/L Cl\textsuperscript{−}, 2.5 mmol/L Ca\textsuperscript{2+}, 8.0 mmol/L Mg\textsuperscript{2+}, 25 mmol/L HCO\textsubscript{3}\textsuperscript{−}, 0.5 mmol/L H\textsubscript{2}PO\textsubscript{4}\textsuperscript{−}, 5.6 mmol/L d-glucose, and 20 g/L dextran (Sigma Chemical Co), with a pH of 7.4 and a partial O\textsubscript{2} pressure of 903 mm Hg when bubbled with 95% O\textsubscript{2} and 5% CO\textsubscript{2}. The perfusate also included 0.9 mmol/L sodium nitroprusside (Sigma), 10 mg/L furosemide (Hoechst Marion Roussel Ltd), and 10 mg/L inulin (Innatec, Lacrovan). The amounts of nitroprusside and furosemide used in the present study were believed to be sufficient to make the kidneys maximally vasodilated and inhibit tubuloglomerular feedback, because the perfused kidneys almost lost their autoregulatory capabilities (see Results). This was also tested in additional experiments in which an additional infusion of 0.07 mg/min acetylcholine did not further reduce renal perfusion pressure.

Thirty minutes after initiating the renal perfusion, collections of urine and measurements of arterial inflow pressure and intrarenal tissue pressure were repeated during 7 or 8 stepwise increments of pressure flow from 3.0 to 19.0 mL/min. The urinary volume was determined gravimetrically. Inulin concentrations in the perfusate and urine were measured by the anthrone method, and the glomerular filtration rate (GFR) was calculated as inulin clearance.

After the final measurement, the perfusion was continued at 3.0 mL/min. Twenty minutes later, 100 μmol/L phenylephrine (Sigma), 1.25 μmol/L [Arg\textsuperscript{1}]vasopressin (Wako Pure Chemical Industries Ltd), and 1.0 μmol/L angiotensin II (Bachem AG) dissolved in Tyrode’s solution were added to the perfusate for another 5 minutes to assess the maximal renal vasoconstrictor response in a half of each group. Then the remaining half of each group, the perfusate was changed to a fixative containing 2% formaldehyde and 0.5% glutaraldehyde in 0.075 mol/L phosphate buffer (pH 7.2), and the kidneys were perfused at 3.0 mL/min for 50 to 60 minutes. In this preparation, perfusion of the fixative did not alter the perfusion pressure, suggesting that there was no effect on the relaxed status of the vasculature. Then the left kidney was removed and placed in 7.4% formaldehyde. The kidneys were embedded in paraffin, sectioned at 3- to 4-μm thickness, and stained with hematoxylin and eosin. At the end of the experiment, the heart was dissected into left and right ventricles and weighed separately.

Estimations of Renal Hemodynamics in Perfused Kidneys

In the maximally vasodilated perfused kidneys, neurohumoral and active autoregulatory control systems are assumed to be inoperative; therefore, the analysis of hemodynamic behavior enables us to perform functional assessments of structural properties in the renal resistance vessels. In the flow-pressure relationship, the slope is the renal vascular resistance at maximal dilatation (ie, minimal renal vascular resistance), which is an accurate index of averaged overall luminal dimensions of the preglomerular and postglomerular vasculature. In the pressure-GFR relationship, the slope reflects the filtration capacity and filtration capability against pressure, respectively.

In the present study, the flow-pressure and pressure-GFR relationships were established by the use of perfusion flow, arterial distending pressure, and GFR at each flow rate to estimate the above-mentioned 3 functional parameters. In these relationships, arterial distending pressure (ie, transmural pressure), calculated as arterial inflow pressure minus renal tissue pressure, was adopted as pressure, because tissue pressure was built up during the perfusion, thereby inhibiting the distension of the vasculature. Maximal renal vascular resistance was also calculated from the data during the renal vasoconstrictor response to vasoactive substances.

Renal Morphological Measurements

Cross-sectional areas of the wall and lumen in the proximal interlobular arteries and glomerular tuft areas were measured with a digitizing tablet (SD-510C, Wacom Co) under a light microscope. The proximal interlobular arteries were defined as being within the inner cortex, branching for 500 μm from the arcuate arteries at the corticomedullary junction. The wall-to-lumen ratio was calculated by dividing the cross-sectional area of the wall by that of the lumen.

Data Analysis and Statistics

All data are expressed as mean±SEM. In renal hemodynamic measurements, inflow volume and GFR were expressed as values divided by the wet weight of the right kidneys. The assumption that...
flow-pressure and pressure-GFR relationships were linear in all individual experiments was tested by using the Pearson correlation coefficient in simple regression analysis. The slope and x-intercept of these relationships were derived from the individual regression lines. The intergroup comparisons of all data were performed by the Student unpaired *t* test. A value of *P*<0.05 was considered statistically significant.

**Results**

Body weight, left ventricle, and right kidney wet weight were greater in DS rats than in DR rats (Table 1). However, there were no differences in mean arterial pressure and pulse rate between the 2 groups. The urinary protein-to-creatinine ratio in DS rats was 4 times that in DR rats.

The relationships between perfusion flow and arterial distending pressure in the maximally vasodilated perfused kidneys are shown in Figure 1. Regression analysis showed a significant linear relationship within each individual experiment, with Pearson correlation coefficients (r*) ranging from 0.96 to 1.00. There was no significant difference in the slope (3.48±0.27 versus 3.77±0.27 mm Hg · mL⁻¹ · min⁻¹ · g kidney wet wt⁻¹) than in DR rats (5.28±0.30 μL · min⁻¹ · g wet kidney wt⁻¹ · mm Hg⁻¹) (*P*<0.01). In contrast, there was no significant difference in the extrapolated intercept of the line with the abscissa (ie, the threshold pressure for initiation of filtration) between the 2 groups (28.5±1.8 mm Hg in DS rats versus 24.6±2.6 mm Hg in DR rats, *P*=NS). Thus, at any given increase of pressure, glomerular filtration was less in DS rats than in DR rats, although glomerular filtering began at a similar distending pressure in the 2 groups. Maximal renal vascular resistance measured under the dosing of vasoconstrictors was greater in DS rats (109±8 mm Hg · mL⁻¹ · min⁻¹ · g kidney wet wt⁻¹) than in DR rats (66±9 mm Hg · mL⁻¹ · min⁻¹ · g kidney wet wt⁻¹) (*P*<0.01).

Table 2 depicts the histological analysis of the renal resistance vessels and glomeruli at maximal vasodilatation. At the interlobular arteries, medial cross-sectional area was significantly greater in DS rats than in DR rats. The internal luminal area was 6% less in DS rats than in DR rats, although the difference was not significant. Consequently, the media-to-lumen ratio was elevated in DS rats compared with DR rats. There were no increases in mesangial matrix and no glomerular sclerosis revealed by microscopy for either group. However, the glomerular area was significantly greater in DS rats than in DR rats, especially at the superficial glomeruli.

**Discussion**

In the perfused kidneys, the slope of the flow-pressure relationship did not differ between DS rats and DR rats. Therefore, the averaged overall luminal dimensions of the

### Table 1. Hemodynamics, Organ Weights, and Urinary Protein Excretion

| Variables                        | DS Rats (n=10)   | DR Rats (n=10) | *P*
|----------------------------------|------------------|----------------|-----
| Body weight, g                   | 393±6            | 332±5          | 0.01|
| Mean arterial pressure, mm Hg    | 126±7            | 122±3          | 0.57|
| Heart rate, bpm                  | 436±23           | 404±12         | 0.24|
| Left ventricle, g/100 g of BW    | 0.223±0.004      | 0.209±0.003    | 0.02|
| Right ventricle, g/100 g of BW   | 0.062±0.002      | 0.060±0.002    | 0.49|
| Right kidney wet weight, g/100 g | 0.453±0.016      | 0.404±0.004    | 0.01|
| Urinary protein/creatinine ratio | 9.02±1.63        | 2.05±0.27      | 0.01|

Values are mean±SEM. BW indicates body weight.

### Table 2. Morphological Characteristics of Preglomerular Vessels and Glomeruli in Maximally Vasodilated Kidneys

| Morphological Variables          | DS Rats (n=5)   | DR Rats (n=5) | *P*
|----------------------------------|-----------------|---------------|-----
| Interlobular artery              |                 |               |     |
| Luminal cross-sectional area, μm²×1000 | 10.49±0.26    | 11.12±0.36    | 0.20|
| Medial cross-sectional area, μm²×1000 | 3.99±0.15       | 3.50±0.17     | 0.05|
| Media/lumen ratio                | 0.39±0.01       | 0.32±0.01     | 0.01|
| Glomerular area, μm²×1000        |                 |               |     |
| Superficial glomeruli            | 13.54±0.53      | 11.56±0.24    | 0.01|
| Juxtaglomerular glomeruli        | 16.90±0.96      | 14.85±0.31    | 0.08|

Values are mean±SEM.
The observation of Sterzel et al. showing that electron microscopy defects leading to a disturbance in glomerular permeability in the proteinuria implies the existence of some undetectable structural glomerular damages on light microscopy. However, the marked glomeruli in DS rats exhibited no abnormalities indicative of little, if any, luminal narrowing.

The maximum contractile response in vascular beds is affected mainly by the averaged maximal contractile strength of the renal vascular wall in relation to its luminal size (ie, wall-to-lumen ratio) if the contractile property is similar in both groups. In the present study, the maximal renal vascular resistance was greater in DS rats than in DR rats. Combined with the hemodynamic data obtained for the vasodilated kidneys, these findings suggest that the wall-to-lumen ratio is greater in the renal vasculature of DS rats probably because of increased wall thickness but not because of alterations in luminal dimension. This notion was also supported by the histological appearance of the proximal interlobular arteries of DS rats, which showed an increase in the wall-to-lumen ratio that was mainly due to the increased wall cross-sectional area. Therefore, expressed in terms of vascular remodeling, the observed vascular structural changes can be described as hypertrophic remodeling with little, if any, luminal narrowing.

In the renal tissues fixed under vasodilated conditions, the glomeruli in DS rats exhibited no abnormalities indicative of glomerular damages on light microscopy. However, the marked proteinuria implies the existence of some undetectable structural defects leading to a disturbance in glomerular permeability in the prehypertensive stage of DS rats. This possibility is supported by the observation of Sterzel et al. showing that electron microscopy detected segmental losses of epithelial foot processes despite no apparent abnormalities detected by light microscopy in the glomeruli of very young (ie, 4-week-old) DS rats. Thus, in DS rats, it is presumed that the structural defects already exist in the glomeruli before the development of hypertension. Possibly, the above glomerular structural defect may provide a basis for the reduction in glomerular permeability and low GFR in the pressure-GFR relationship. The observed glomerular hypertrophy also appears to be a compensatory adjustment to offset the loss of glomerular permeability by increasing the glomerular surface area.

There are several aspects of the design of the present study that require comment. In the present study, the lumen of the renal vasculature was hemodynamically assessed by using the in vitro maximally dilated whole-kidney perfusion technique. This methodology has already been applied for measurements of the vascular structure in SHR by Göthberg and colleagues and their results in the vasodilated perfused kidney model are in good agreement with those obtained by other techniques. In contrast to histological analysis, this technique is accurate and sensitive enough to detect the alterations of lumen in small resistant vessels, because vascular resistance varies inversely with the fourth power of the radius, according to the Poiseuille’s law. It can also determine whether the changes of the lumen are confined to the preglomerular or postglomerular circulation. However, our technique does not allow us to conclude whether the luminal changes are in the afferent arteriole, larger upstream vessels, or both or whether the changes are in the cortical or medullary vessels. In addition, renal denervation or adding of spironolactone to the perfusate might have been required to exclude completely the possible residual influences of renal nerves or aldosterone on the measurements. In the histological analysis, renal tissues were perfusion-fixed under maximally vasodilated conditions to minimize the artificial vasoconstriction induced by fixative during the perfusion-fixation process.

Our data of the renal vascular structure in DS rats are clearly different from those reported in the prehypertensive or early hypertensive SHR. In the vasodilated perfused kidneys of SHR compared with those of age-matched normotensive controls, the pressure-GFR relationship shows a slightly greater x-intercept without changes in the slope of the line. This hemodynamic observation in the vasodilated kidneys indicates that the glomerular filtration capability against pressure is maintained normally in SHR, although the preglomerular-to-postglomerular vascular resistance ratio is elevated. Therefore, it seems likely that renal structural alterations precede frank hypertension in both DS rats and SHR, but the most affected sites are different between them. In particular, a structural defect in filtering is present in the glomeruli of DS rats but not SHR. The other hand, vascular narrowing occurs in the preglomerular resistance vessels of SHR but not DS rats.

Accordingly, on the basis of the structural properties, we hypothesize the following sequence of events with age in the kidneys of DS rats: (1) The reduced glomerular filtration capacity and the elevation of the wall-to-lumen ratio in the renal vasculature occur as early events before the development of hypertension. The former could reduce renal excretory function, which leads to volume expansion. The latter will tend to enhance vascular contractile response if the neurohumoral influences to the kidneys remain unchanged. This enhanced renal vasoconstriction could lower glomerular pressure and aggravate the reduction in renal excretory capability further. (2) As a result, systemic pressure will increase to offset the reduced renal excretory functions. The glomerular area might also increase to compensate for the decreased glomerular filtration and thereby increase the filtration fraction. (3) Thereafter, vascular hypertrophy could progress with age by pressure loading, thus encroaching into the lumen, as reported for the hindquarter vasculature. Furthermore, this vascular narrowing will accentuate the development of hypertension if DS rats are maintained on a low salt diet or will lead to a malignant-type hypertension on a high salt diet. In addition, it could be also hypothesized that in DS rats, intraglomerular pressure might increase concomitantly during the development of hypertension because of the less pronounced vascular narrowing at the preglomerular vasculature than is found in SHR. However, to confirm the above hypothesis, further studies are needed concerning the effects of salt loading or aging on the renal
vasculature. It must be also emphasized that such intrarenal hemodynamics is confounded or modified by the alterations of other factors, including intrarenal autoregulation mechanism, vascular reactivity, and endothelial function.41–44

In conclusion, our data reveal that hypertrophic remodeling without a luminal narrowing at the preglomerular resistance vessels and a permeability defect in filtering at the glomeruli exist in the prehypertensive stage of DS rats. The observed renal structural characteristics may be involved in the genesis of glomerular hypertension in DS rats.

References


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