Renal vascular dysfunction precedes the development of renal damage in the hypertensive Fawn-Hooded rat

Peter Ochodnický,1 Robert H. Henning,2 Hendrik J. Buikema,2 Dick de Zeeuw,2 Abraham P. Provoost,3 and Richard P. E. van Dokkum2

1Faculty of Pharmacy, Department of Pharmacology and Toxicology, Comenius University Bratislava, Bratislava, Slovakia; 2Department of Clinical Pharmacology, University Medical Center Groningen and Groningen Institute for Drug Exploration, University of Groningen, Groningen; and 3Department of Pediatric Surgery, Erasmus Medical Center, Rotterdam, The Netherlands

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Ochodnický P, Henning RH, Buikema HJ, de Zeeuw D, Provoost AP, van Dokkum RP. Renal vascular dysfunction precedes the development of renal damage in the hypertensive Fawn-Hooded rat. Am J Physiol Renal Physiol 298: F625–F633, 2010. First published December 9, 2009; doi:10.1152/ajprenal.00289.2009.—It is unknown whether generalized vascular dysfunction precedes the development of kidney disease. Therefore, we studied myogenic constriction and endothelium-mediated dilatory responses in two inbred Fawn-Hooded (FHH) rat strains, one of which spontaneously develops hypertension, proteinuria, and glomerulosclerosis (FHH), whereas the other (FHL) does not. Small renal, mesenteric resistance arteries and thoracic aorta isolated from FH rats before (7 wk old) and after the development of mild proteinuria (12 wks old) were mounted in perfused and isometric set-ups, respectively. Myogenic response, endothelium-dependent relaxation, and the contribution of endothelium-mediated dilatory compounds were studied using their respective inhibitors. Myogenic reactivity was assessed constructing pressure-diameter curves in the presence and absence of calcium. At the age of 7 wk, renal arteries isolated from kidneys of FHH rats developed significantly lower myogenic tone compared with FHL, most likely because of excessive cyclo-oxygenase 1-mediated production of constrictive prostaglandins. Consequently, young FHH demonstrated reduced maximal myogenic tone (22 ± 4.8 vs. 10.8 ± 2.0%, P = 0.03) and the peak myogenic index (−6.9 ± 4.8 vs. 0.6 ± 0.8% mm Hg, P = 0.07 for FHL vs. FHH, respectively). Active myogenic curves obtained in mesenteric arteries isolated from 7-wk-old rats did not differ between either strain, demonstrating a similar level of systemic myogenic tone in FHL and FHH rats. Therefore, before any renal end-organ damage is present, myogenic response seems selectively impaired in renal vasculature of FHH rats. Aortic reactivity did not differ between FHL and FHH at the time points studied. The present study shows that vascular dysfunction in both small renal and systemic arteries precedes renal end-organ damage in a spontaneous model of hypertension-associated renal damage. These early vascular changes might be potentially involved in the increased susceptibility of FHH rats to renal injury.

myogenic response; endothelial dysfunction; Fawn-Hooded rat; chronic kidney disease; hypertension; nitric oxide; endothelium-derived hyperpolarizing factor; prostaglandins

IN CHRONIC KIDNEY DISEASE, a progressive deterioration in renal function is associated with abnormal regulation of arterial tone (19, 34). A growing body of evidence indicates that even patients with microalbuminuria exhibit generalized endothelial dysfunction in both renal and systemic vascular beds (4, 22). In fact, it has been suggested that endothelial dysfunction might precede the development of microalbuminuria (1, 29). Endothelium-dependent relaxant responses are mediated by the release of vasoactive factors, such as nitric oxide (NO), cyclooxygenase (COX)-derived prostaglandins (PGs), and the yet unidentified endothelium-derived hyperpolarizing factor (EDHF) (31). We have previously shown that the interindividual variability in endothelium-dependent reactivity of intra-renal arteries, including NO-, PG-, and EDHF-mediated responses, among healthy rats of an outbred Wistar rat strain predicts their susceptibility to subsequent renal damage induced by renal mass reduction (11) or nephrotoxic drug (20). This suggests that endothelial function might be one of the factors governing the susceptibility to experimental renal end-organ damage.

In addition to experimental models, specific inbred animal strains have been described spontaneously developing progressive renal disease (17). The Fawn-Hooded hypertensive (FHH) rat is a model of genetic renal damage in which five genes for renal failure were identified and shows impairment of renal autoregulation and renal myogenic response in contrast to its normotensive counterstrain, the FHL rat. In particular, one of the genes (Rf-1) has been implicated in renal blood flow autoregulation. The homologous region of Rf-1 has been identified on human chromosome 10 linked to markers of end-stage renal disease, stressing the importance of this model in scientific research and screening regarding a wide variety of human forms of renal disease involving vascular dysfunction. FHH rats spontaneously develop moderate hypertension, proteinuria, and severe glomerulosclerosis at a young age, subsequently followed by progressive renal failure (14, 23, 28). In contrast, FH rats with low blood pressure (FHL) seem to be resistant to the development of hypertension and renal damage (23). It has been proposed that altered vascular smooth muscle mediated reactivity to intraluminal pressure, termed myogenic response, in preglomerular arteries might be responsible for different sensitivity of these strains to renal injury. Specifically, the Fawn-Hooded rat showed impaired autoregulation of renal blood flow and intraglomerular pressure (8). Yet, the data comparing renal myogenic response in FHL and FHH rats provide inconsistent results (8, 24), whereas the role of endothelial reactivity is unknown.

In the present study, we aim to define the role of vasomotor changes in the course of spontaneous hypertension-associated renal disease. First, we explored whether renal vascular dysfunction precedes the development of renal damage. To this...
end, in animals prone (FHH) and resistant (FHL) to renal damage, we compared endothelium-mediated (NO-, PGs, and EDHF-dependent) and myogenic responses of small renal arteries at an early age, before the appearance of renal damage. In addition, we explored whether early renal vascular changes reflect generalized vasomotor dysfunction by studying endothelial and myogenic responses in small resistance (mesenteric) and large conduit arteries (aorta). Finally, we evaluated the observed vascular changes in time, by investigating vasomotor reactivity in animals at the older age, when mild proteinuria had already developed.

MATERIALS AND METHODS

Animals and in vivo measurements. Experiments were performed in young male FHL and FHH rats at week 7 and week 12 after birth (n = 9–12/strain and per time point). In FHH rats, this time frame represents the ages in which no and minor proteinuria is detected, respectively. All animals were bred at the animal facilities of Erasmus University, Rotterdam, the Netherlands, and housed under standard conditions in an animal facility at the University of Groningen, the Netherlands, receiving food and water ad libitum. Shortly before reaching their target age, animals were put in metabolic cages to measure fluid intake and urine output. Urinary protein excretion was determined by nephelometry (Dade Behring III, Mannheim, Germany) in 24-h urine samples. Subsequently, animals were anesthetized by 2% isoflurane in N₂O-O₂ (2:1), the right carotid artery was cannulated, and systolic and diastolic blood pressure were measured by a pressure transducer catheter (Millar Instruments) in the aortic root. After these measurements, blood was drawn via an anal aorta. Subsequently, mesenterium, kidneys, and thoracic aorta were harvested for the analysis of vascular function and end-organ damage. All animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Committee for Animal Experiments of the University of Groningen.

Determination of renal damage. Plasma and urine creatinine were measured by means of a photometric assay with the Jaffé method without deproteinization (DiaSys Diagnostic Systems, Holzheim, Germany), and creatinine clearance was calculated as creatinine clearance = (urine creatinine × urine flow)/(plasma creatinine × body wt). Paraffin-embedded kidneys were cut in 3-μm sections and stained with periodic acid Schiff, and the incidence of focal glomerulosclerosis (FGS) score was microscopically evaluated according to standard procedures as described previously (6). Vascular reactivity of small renal and systemic resistance arteries. Small renal (interlobar) arteries (~200–250 μm) and third-order branches of superior mesenteric arteries (~300 μm) were cleaned from perivascular tissue and transferred to an arteriograph system for pressurized arteries (Living System Instrumentation, Burlington, VT) as described previously (8). Artery segments were cannulated on glass micropipettes, and the vessel chamber was continuously recirculated with warmed (37°C) and oxygenated (5% CO₂ in O₂) Krebs solution with a pH of 7.4. An inverted light microscope attached to a video camera and video dimension analyzer was used to continuously register lumen diameter.

Myogenic reactivity of small renal and systemic resistance arteries. Intraluminal pressure was set at 80 mmHg, and arteries were allowed to equilibrate for 40 min and checked by a single dose of phenylephrine (PE, 3 × 10⁻⁷ mol/l) and acetylcholine (ACh; 3 × 10⁻⁶ mol/l) for smooth muscle and endothelium viability, respectively. Following a wash out, intraluminal pressure was decreased to 20 mmHg, and myogenic reactivity was studied by obtaining active pressure-diameter curves over a pressure range of 20–160 mmHg in steps of 20 mmHg. Each pressure step was maintained for 5 min to reach the stable contractile response. Following the myogenic protocol, preparations were washed with Krebs solution and employed for investigation of endothelial function as described below. Thereafter, calcium containing Krebs solution was exchanged for calcium-free Krebs solution supplemented with ethylene glycol-bis-(β-aminoethyl ether) tetraacetic acid (EGTA, 2 mmol/l), and passive pressure-diameter curves were obtained over the same 20- to 160-mmHg pressure range.

To explore the role of endothelium in myogenic tone regulation, the effect of endothelium removal was investigated in additional renal arteries (n = 10 for each strain) isolated from 12-wk-old animals. Endothelium was removed by perfusing the preparation with 5 ml of air, and endothelial removal was confirmed by the absence of a dilute response to ACh (3 × 10⁻⁵ mol/l) following a submaximal preconstriction with PE (3 × 10⁻⁷ mol/l). Subsequent to the washout, active and passive myogenic curves were recorded as described above.

Endothelium-dependent relaxation of small renal and systemic resistance arteries. Following the measurement of active myogenic curves, the vessels were allowed to recover to their original diameter at 80 mmHg, and arteries were washed and stabilized for 20 min. Because the level of spontaneous tone was not sufficient for the subsequent relaxation studies, arteries were preconstricted with PE (3 × 10⁻⁷ to 10⁻⁶ mol/l) to 50–60% of initial baseline diameter. Endothelium-dependent relaxation was assessed by administering cumulative doses of ACh (10⁻⁹ to 3 × 10⁻⁵ mol/l) to the recirculating bath. After the construction of a full ACh concentration-response curve and wash out, the response to ACh was studied in the same artery in the presence of indomethacin (10⁻⁵ mol/l) to inhibit PG production. Subsequently, the same procedure was repeated in the combined presence of indomethacin and the NO production inhibitor Nω-nitro-l-arginine (l-NMMMA, 10⁻⁴ mol/l). In some arteries of both vascular types (FHL and FHH, n = 4 each), we found that this remaining relaxation in the presence of indomethacin and l-NMMMA was caused by the release of EDHF, since it was completely attenuated by the combination of the potassium channels blockers charybdoxin (10⁻⁷ mol/l) and apamin (5 × 10⁻⁷ mol/l). By analyzing the above-mentioned protocols of endothelium-dependent relaxation, the contribution of all three mediators (PGs, NO, and EDHF) to endothelial relaxation was calculated as a difference between area under the curve of respective ACh concentration-response curves.

Involvement of cyclooxygenase pathway in endothelium-dependent contractions of small renal arteries. To investigate the underlying mechanisms of prostanooid-dependent endothelium-mediated contractions observed in small renal arteries of FHH rats, in separate set of arteries (n = 6), ACh concentration-responses were obtained in the presence of either the COX-1 selective inhibitor valeryl salicylate (VAS, 10⁻⁴ mol/l), the COX-2 selective inhibitor NS-398 (10⁻⁶ mol/l), the thromboxane (TX) A₂/PGF₂α receptor antagonist SQ-29548 (10⁻⁶ mol/l), or the superoxide scavenger superoxide dismutase (50 U/ml).

General smooth muscle reactivity of small renal and systemic arteries. Additional arteries were used to control for the potential variation in depolarization-and receptor-mediated smooth muscle reactivity. After a stabilization period, concentration-response contractile curves were obtained using KCl (20–120 mmol/l) and PE (10⁻⁸ to 10⁻⁵ mol/l) with a wash out period between the protocols. Additionally, concentration-response curves to the direct smooth muscle vasodilator sodium nitroprusside (SNP, 10⁻⁹ to 3 × 10⁻⁵ mol/l) were constructed after submaximal preconstriction with PE (3 × 10⁻⁷ to 10⁻⁶ mol/l).

Vascular reactivity of isolated aortic rings. The thoracic aorta was cleaned from the connective tissue and cut into 2-mm rings, which were mounted in isotonic contraction organ baths filled with aerated, warmed Krebs solution and subjected to 14 mN preload. After a 1-h stabilization period, arteries were stimulated by KCl (60 mmol/l) to check their viability, washed out, and preconstricted submaximally by 10⁻⁴ mol/l PE. Endothelium-dependent relaxation was investigated similarly to the protocol performed in perfused small arteries, e.g.,
concentration-response curves to acetylcholine (10⁻⁹ to 10⁻⁵ mol/l) were obtained in the absence and subsequently in the presence of indomethacin (10⁻⁵ mol/l) and indomethacin + L-NMMA (10⁻⁴ mol/l) to investigate the contribution of PGs, NO, and EDHF to endothelium-mediated relaxation. In other rings, dose-response curves to PE (10⁻⁹ to 10⁻⁵ mol/l) were followed by measurements of reactivity to SNP (10⁻¹⁰ to 10⁻⁵ mol/l).

Chemicals. Krebs solution had a following composition (in mM): 120.4 NaCl, 5.9 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 25.0 NaHCO₃, 1.2 NaH₂PO₄, and 11.5 glucose. All of these compunds were purchased from Merck (Darmstadt, Germany). VAS, NS-398, and SQ-29548 were purchased from Cayman Chemical (Ann Harbor, MI). All other drugs were obtained from Sigma-Aldrich Chemie, the Netherlands. They were dissolved either in ethanol (VAS, NS-398, SQ-29548) or in deionized water and diluted with Krebs solution. Stock solution for indomethacin was prepared in 96 mmol NaHCO₃.

Statistical analysis and calculations. Data are expressed as means ± SE. Myogenic tone, describing myogenic behavior of an artery at a given pressure, was expressed as the percent decrease in active diameter from the maximally dilated (passive) diameter determined at the same pressure in calcium-free/EGTA solution, i.e., myogenic tone (%) = 100 [(Dₑ - Dₒ)/Dₑ], where D is the diameter in calcium-free (Dₑ) or calcium-containing (Dₒ) Krebs. The myogenic index, describing myogenic reactivity of an artery in response to a pressure change, i.e., the slope of active pressure-diameter relationship, was calculated for every 20 mmHg pressure step (ΔP) as a percentage change in corresponding active diameter D, i.e., myogenic index (%/mmHg) = 100 [(ΔD/ΔP)]. For each individual artery, maximal myogenic tone and peak myogenic index were determined from all the pressures and pressure steps studied, respectively. Concentration-response curves to the vasoconstrictors KCl and PE were calculated as a percentage change from baseline artery diameter and from maximal KCl response for small arteries and aorta, respectively. Concentration-response curves of the vasodilators ACh and SNP were expressed as a percentage of preconstriction to PE. The curves were characterized by the maximal relaxation (Emax) and the negative logarithm of ACh molar concentration causing half-maximal relaxation (pD₂). Full myogenic and concentration-response curves of ACh and SNP were compared by ANOVA for repeated measures followed by Bonferroni post hoc test for multiple comparisons. Group comparison of animal and vascular parameters was performed by unpaired Student’s t-test. Differences were considered significant if P < 0.05 (2-tailed).

RESULTS

Animal characteristics. Characteristics of FHL and FHH rats in both experimental periods are given in Table 1. In 7-wk-old FHL and FHH rats, no renal damage was present, as evidenced by similar levels of proteinuria, FGS, plasma creatinine, and creatinine clearance. Both systolic and diastolic blood pressure were marginally elevated in FHH rats compared with FHL. Later (5 wk), FHH rats developed significant proteinuria, without an increased incidence of FGS or loss of renal function. This suggests that, in 12-wk-old FHH rats, mild renal damage is present. In contrast to proteinuria, blood pressure did not increase in either strain compared with week 7. Additional animals (n = 10 for each strain) were followed for 26 wk to confirm development of renal damage. At this age, hypertension, profound proteinuria, and structural damage were present in FHH rats, but not in FHL rats (data not shown).

Myogenic reactivity is selectively impaired in the renal vasculature of FHH rats before the development of renal end-organ damage. At the age of 7 wk, passive diameters of small renal arteries did not differ between FHL and FHH rats in the pressure range studied (Fig. 1A). However, as evidenced by the differences in active curves (Fig. 1A), renal arteries isolated from kidneys of FHH rats developed significantly lower myogenic tone compared with FHL (Fig. 2A). Consequently, young FHH demonstrated reduced maximal myogenic tone (22 ± 4.8 vs. 10.8 ± 2.0%, P = 0.03) and the peak myogenic index (−6.9 ± 4.8 vs. 0.6 ± 0.8%/mmHg, P = 0.07 for FHL vs. FHH, respectively). In contrast to small renal arteries, active myogenic curves obtained in mesenteric arteries isolated from 7-wk-old rats did not differ between either strain (Fig. 1C), demonstrating a similar level of systemic myogenic tone in FHL and FHH rats (Fig. 2C). Therefore, before any renal end-organ damage is present, the myogenic response seems impaired selectively in the renal vasculature of FHH rats.

Beside basing the myogenic response on the most reactive single data point, it might be more representative to take the slope of the change in diameter over a defined pressure range. For the interlobar artery in Fig. 1 from 80 or 100 to 160 mmHg, it suggests impaired autoregulation in 7-wk-old FHH vs. FHL. Moreover, autoregulation is slightly better in both groups at the age of 12 wk and evident from a constancy of diameter over the 100- to 160-mmHg pressure range, for FHH and FHL. Diameter may actually fall to a significant extent in FHL.

When similar slope analyses are performed for pressure-dependent changes in diameter of the third-order mesenteric artery and aorta, inspection of the data in Fig. 1 also suggests

Table 1. In vivo characteristics of FHL and FHH rats showing either no renal (7 wk of age) and minor renal (12 wk of age) end-organ damage

<table>
<thead>
<tr>
<th></th>
<th>No Renal Damage</th>
<th>Minor Renal Damage</th>
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<tbody>
<tr>
<td></td>
<td>FHL</td>
<td>FHH</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>215 ± 3</td>
<td>211 ± 3</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>118 ± 3</td>
<td>130 ± 4*</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>76 ± 3</td>
<td>86 ± 3*</td>
</tr>
<tr>
<td>Fluid intake, ml/24 h</td>
<td>33 ± 5</td>
<td>40 ± 3</td>
</tr>
<tr>
<td>Urine output, ml/24 h</td>
<td>19 ± 2</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>Proteinuria, mg/24 h</td>
<td>19 ± 1</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>Plasma creatinine, μmol/l</td>
<td>51 ± 3</td>
<td>50 ± 2</td>
</tr>
<tr>
<td>Creatinine clearance, ml·min⁻¹·100 g body wt⁻¹</td>
<td>7.4 ± 0.4</td>
<td>6.2 ± 0.2</td>
</tr>
<tr>
<td>Kidney wt, g</td>
<td>1.1 ± 0.03</td>
<td>1.02 ± 0.03</td>
</tr>
<tr>
<td>FGS score, %</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
</tr>
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</table>

Values are means ± SE. FHL, Fawn-Hooded rat with low blood pressure; FHH, Fawn-Hooded rat with hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; FGS, focal glomerulosclerosis. P < 0.01 vs. FHL of the same age (*) and vs. the same strain at age of 7 wk (†).
that the myogenic response is stronger in mesenteric vs. interlobar arteries at 7 wk of age. The slopes of diameter changes seem to be similar in mesenteric and interlobar arteries at 12 wk of age.

Selective renal impairment of myogenic reactivity is more pronounced after the development of proteinuria. At 12 wk of age, small renal arteries from Fawn-Hooded rats showed a marked difference between active and passive myogenic curves (Fig. 1B), developing more pronounced myogenic tone (Fig. 2B) compared with 7-wk-old animals (Fig. 2A). In contrast, the level of myogenic tone in 12-wk-old FHH rats remained minimal and significantly different from FHL animals (Fig. 2B), as reflected by markedly impaired maximal myogenic tone and peak myogenic index in FHH rats compared with FHL (Table 2). In small mesenteric arteries at 12 wk, active myogenic curves (Fig. 1D) and myogenic tone (Fig. 2D) development were nonsignificantly higher in FHL compared with FHH. As a result, maximal myogenic tone and peak myogenic index in mesenteric arteries (Table 2) was not significantly different between FHL and FHH rats. Therefore, selective impairment of myogenic reactivity in small renal arteries is even more pronounced after the development of renal damage.

To investigate whether impaired contractile ability of small arteries is confined to myogenic stimuli or a general impairment of vascular contraction, we investigated depolarization- and receptor-mediated contraction to KCl and PE, respectively. Depolarization- and receptor-mediated contractile ability in both vascular beds studied were similar between FHL and FHH rats, as evident from the characteristics of the concentration-response curves to KCl and PE shown in Table 2. To explore whether the endothelium plays a role in the impairment of myogenic tone in renal arteries of FHH rats, we repeated the myogenic protocol after removal of the endothelium. As shown in Fig. 3, removal of the endothelium did not attenuate the differences in myogenic reactivity between FHL and FHH rats.

Renal and systemic endothelial dysfunction in FHH rats before the development of renal end-organ damage. ACh relaxed small renal arteries isolated from 7-wk-old FHL rats, resulting in a monophasic concentration-response curve (Fig. 4A). In FHH rats, however, a biphasic curve was observed as higher concentrations of ACh induced a contractile response (Fig. 4A). In contrast to the renal artery, ACh incubation resulted in monophasic concentration-response curves in both FHL and FHH rats in small mesenteric arteries, although the curve was shifted to the right in FHH rats (Fig. 4C). Unlike in small arteries, no difference between ACh-induced relaxations of the two strains was observed in aortic rings (Fig. 4E). All observed alterations specifically indicated the presence of endothelial dysfunction, since no differences were found in the reactivity to the endothelium-independent vasodilator SNP in any vascular bed between FHL and FHH rats (Table 2).

Progression of systemic endothelial dysfunction in FHH rats after the development of proteinuria. At the age of 12 wk, animals showed a similar pattern of endothelial dysfunction in the investigated vascular beds as observed at the age of 7 wk. In 12-wk-old animals, endothelial dysfunction was present in small renal (Fig. 4B) and mesenteric (Fig. 4D) arteries, but not in aorta (Fig. 4F). In small mesenteric artery, endothelial dysfunction in FHH was aggravated when compared with 7-wk-old rats, as evidenced by a further shift of the response curve to the right (Fig. 4D). SNP-induced relaxation remained unchanged in all vascular beds.

Heterogeneous mechanisms underlying endothelial dysfunction in FHH rats. To explore the mechanisms responsible for the endothelial dysfunction in studied vascular beds, we con-
constructed an ACh concentration-response curve in the presence of the inhibitors of endothelium-derived vasodilatory mediators in both 7- and 12-wk-old animals. Because similar curves were obtained at both time points, for reasons of clarity, only data from week 12 are presented in Fig. 5. Indomethacin, an inhibitor of COX, completely reversed endothelium-dependent contractions associated with higher doses of ACh in small renal arteries of FHH rats (Fig. 5B), whereas it had no significant effect in FHL rats (Fig. 5A). Additional blockade of NO by L-NMMA resulted in significant attenuation of Ach vasodilation in renal arteries of FHH rats (Fig. 5B), while affecting the relaxation to a lesser extent in FHL animals (Fig. 5A). The remaining Ach relaxation was completely blocked by the combined application of potassium channel inhibitors charybdotoxin and apamin, suggesting this response is mediated by EDHF (Fig. 5, A and B).

Indomethacin did not affect the endothelial dilation in either FHL or FHH rats in small mesenteric artery (Fig. 5, C and D). Additional block of NO led to a small right shift of the curve in FHL (Fig. 5C), with an even less pronounced effect in FHH (Fig. 5D). Similar to renal vessels, the remaining relaxation was completely inhibited by charybdotoxin and apamin (Fig. 5, C and D).

In aorta, the Ach-induced response was not altered in the presence of indomethacin, whereas it was almost completely attenuated by NO blockade. No differences were observed between FHL and FHH rats (Fig. 5, E and F).

Based on these observations, the contribution of the principal endothelial mediators (PGs, NO, EDHF) to endothelial function was calculated as the area under the curve in Fig. 5 (data not shown). The release of constrictive PGs seems responsible for endothelial dysfunction in small renal arteries of FHH before the development of renal damage, as indicated by the negative value of the PG contribution. At the same time, PGs do not play any role in endothelial dysfunction in the mesenteric artery, in which, rather, a reduction in EDHF is found. In aorta, no changes in endothelial mediators could be detected.

After the development of renal damage, endothelial dysfunction persists in FHH rats with similar mechanisms involved. Renal arteries showed comparable production of contractile PGs as observed before the development of renal damage. In contrast, mesenteric arteries displayed even more pronounced loss of EDHF-mediated relaxation compared with 7-wk-old FHH rats. No significant changes were observed in the responses of aorta. Additionally, small renal arteries of FHH rats with renal damage rely relatively more on NO-mediated vasodilation than FHL rats. Taken together, the results indicate that nearly all of the dilator response to Ach is mediated by EDHF/EETs in the renal and mesenteric arteries while it is mediated by NO in the aorta.

Mechanisms underlying endothelium-mediated contractions in renal arteries of FHH rats. To identify the mechanisms underlying the Ach-mediated indomethacin-sensitive contractile response in renal arteries of FHH rats, additional experiments were performed in 12-wk-old rats. As shown in Fig. 6, endothelium-dependent contractions to Ach are reversed to relaxations in the presence of COX-1 inhibitors but unaffected by COX-2 inhibitors. Furthermore, the contractions were blunted by a TXA2/PGHS receptor antagonist and by superoxide dismutase, identifying the target receptor of the COX-1-derived prostanoids and suggesting involvement of reactive oxygen species in this response, respectively.
### DISCUSSION

The present study reports selectively impaired myogenic contractility and COX-1-dependent endothelial dysfunction of small renal arteries in an inbred rat model of spontaneous renal disease (FHH) compared with its disease-resistant counterpart (FHL) before the manifestation of renal damage. In systemic resistance mesenteric arteries of FHH, an EDHF-dependent endothelial dysfunction was found. Therefore, heterogeneous vascular dysfunction is present in the FHH before the development of end-organ damage.

A progressive pattern of spontaneous hypertension-associated renal disease in young Fawn-Hooded rats was characterized by a minor elevation in blood pressure in 7-wk-old FHH rats compared with FHL, and markers of renal damage did not differ, while blood pressure did not further increase in FHH rats over the additional 5 wk of the other study group. A marked elevation in proteinuria was observed in accord with previous observations (7, 27, 33), as opposed to FHL (23). These data confirm that FHH rats studied at an early age provide a useful model to investigate the role of vascular changes in the development of spontaneous renal damage.

**Relation between myogenic tone and development of organ damage.** Showing impairment of myogenic reactivity in FHH before the development of proteinuria, the present study extends observations by Van Dokkum et al. (8) showing lower myogenic constriction in the renal interlobular artery of proteinuric FHH rats compared with FHL rats. Interestingly, myogenic reactivity increases with age in FHL, whereas this increase is absent in FHH. This suggests that myogenic mechanisms in FHH rats fail to adapt to increased hemodynamic load during the progression of hypertension-associated renal disease. In accord, elevated intraglomerular capillary pressure at the age of 7 wk (28) and attenuated autoregulation of renal blood flow in 12-wk-old FHH (5) were found. The latter was restored by the transfer of a specific region of chromosome 1 from Brown-Norway rats to FHH, which also leads to normalization of proteinuria (18). Based on maximal change, we conclude that renal autoregulation improves with age in FHL but is absent in 12-wk-old FHH. The collective data for diameter changes in 12-wk-old FHH (Fig. 1B), however, show that there is some ability to develop myogenic tone and seems to be quite sensitive to the initial pressure to which an artery responds to a change in diameter from passive to active. We previously showed that variation in renal endothelial function of healthy rats from an outbred Wistar strain predicts their susceptibility to the development of renal end-organ damage after renal mass reduction (11) and combined unilateral nephrectomy and myocardial infarction (21). In agreement with these observations, renal endothelial function in FHH is already impaired before the development of renal damage, indicating that the endothelium may actively participate in the susceptibility to end-organ damage. Given these observations, we surmise that subtle impairments in renal myogenic tone contribute to progression and maintenance of renal disease.

**Possible mechanism and selectivity of impaired renal myogenic tone.** The primary mechanism underlying endothelial dysfunction in renal arteries of FHH rats is COX-1-mediated endothelium-dependent contraction resulting from excessive production of vasoconstrictive endoperoxides and/or TXs and superoxide radical. Interestingly, early hyperfiltration in FHH rats was reported to coincide with an excessive urinary excretion of contractile PGs (15), suggesting the role of PGs in the development of spontaneous renal injury. However, alternatively, COX-mediated renal endothelial dysfunction may be associated with the development of spontaneous hypertension (30), since it is present at an early, prehypertensive stage in the spontaneously hypertensive rat.

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### Table 2. Characteristics of vasoreactivity of small renal, small mesenteric arteries and aorta isolated from FHL and FHH rats at the age of 12 wk

<table>
<thead>
<tr>
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<th>FHL</th>
<th>FHH</th>
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<tr>
<td><strong>Renal artery</strong></td>
<td></td>
<td></td>
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<tr>
<td>Contractility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{\text{max}}, %$</td>
<td>68 ± 4</td>
<td>71 ± 3</td>
</tr>
<tr>
<td>$pD_2$</td>
<td>1.33 ± 0.08</td>
<td>1.40 ± 0.04</td>
</tr>
<tr>
<td>PE</td>
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<tr>
<td>$E_{\text{max}}, %$</td>
<td>68 ± 4</td>
<td>70 ± 3</td>
</tr>
<tr>
<td>$pD_2$</td>
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<td>6.8 ± 0.2</td>
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<tr>
<td>Myogenic</td>
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<tr>
<td>$E_{\text{max}}, %$</td>
<td>34 ± 5</td>
<td>16 ± 2*</td>
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<tr>
<td>PMI, %/mmHg</td>
<td>−3.2 ± 1.0</td>
<td>−0.9 ± 0.4*</td>
</tr>
<tr>
<td><strong>Mesenteric artery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contractility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{\text{max}}, %$</td>
<td>66 ± 4</td>
<td>60 ± 4</td>
</tr>
<tr>
<td>$pD_2$</td>
<td>6.5 ± 0.1</td>
<td>6.8 ± 0.2</td>
</tr>
<tr>
<td>PE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{\text{max}}, %$</td>
<td>88 ± 3</td>
<td>86 ± 3</td>
</tr>
<tr>
<td>$pD_2$</td>
<td>6.9 ± 0.2</td>
<td>6.9 ± 0.2</td>
</tr>
<tr>
<td><strong>Aorta</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contractility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{\text{max}}, %$ of KCl</td>
<td>75 ± 5</td>
<td>78 ± 5</td>
</tr>
<tr>
<td>$pD_2$, % of KCl</td>
<td>6.7 ± 0.1</td>
<td>6.7 ± 0.1</td>
</tr>
<tr>
<td>Relaxation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{\text{max}}, %$</td>
<td>56 ± 4</td>
<td>57 ± 8</td>
</tr>
<tr>
<td>$pD_2$</td>
<td>6.6 ± 0.1</td>
<td>6.5 ± 0.1</td>
</tr>
<tr>
<td>SNP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{\text{max}}, %$</td>
<td>98 ± 1</td>
<td>100 ± 1</td>
</tr>
<tr>
<td>$pD_2$</td>
<td>7.9 ± 0.1</td>
<td>7.7 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. Shown are parameters of response curves to KCl, α-agonist phenylephrine (PE), increased intraluminal pressure (myogenic tone), endothelium-dependent vasodilator acetylcholine (ACh), and endothelium-independent vasodilator sodium nitroprusside (SNP). $E_{\text{max}}$, maximal contractile response in %baseline vascular diameter (contractility) or in % of preconstriction (relaxation); $pD_2$, negative logarithm of the molar concentration of agonist causing half of the maximal responses; PMI, peak myogenic tone, maximal slope of active myogenic curve (%/mmHg). *$P < 0.05$. 

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SHR, a strain relatively resistant to the development of renal injury (2), possibly being a hypertension-driven renal disease as opposed to a renally initiated hypertension in FHH. Interestingly, although endothelium-dependent contraction is confined exclusively to the renal vasculature in FHH, it is also found in systemic resistance arteries and aorta in SHR (10, 13, 37). Nevertheless, the implications of this observation in defining the role of early renal endothelium-dependent contractions in the pathophysiology of hypertension-associated renal damage in FHH rats remain unclear.

Because receptor- or depolarization-mediated contraction were intact in renal artery of FHH, impairment of renal myogenic activity in FHH seems localized upstream in its signaling cascade, e.g., in mechanotransduction. Only few studies reported heterogeneity of myogenic mechanisms among vascular beds (15), and differential mechanisms have not been characterized yet. Several mechanosensitive ion channels and integrins may be involved in a stretch-induced mechanotransduction, whereas other factors have been implicated in the myogenic signal transduction, including activation of the calmodulin/myosin light chain kinase pathway, protein kinase C, mitogen-activated protein kinases, several potassium channels, and cytochrome P-450-derived metabolites of arachidonic acid (20-HETE, EETs), the latter being EDHF in the renal vasculature (30). Altered activity of any of these components may underlie the impairment of renal myogenic constriction in FHH rats, although several of these mechanisms participate also in receptor- or depolarization-mediated contraction. Since these contractions were intact, the affected mechanism in FHH seems rather specific for the stretch-induced reactivity, such as mechanotransduction. Moreover, the defect is independent of basal reactivity of endothelium, since impairment of myogenic constriction in FHH persisted after removal of the endothelium.

Involvement of NO. Reduced bioavailability of NO is considered to be a key mechanism for renal endothelial dysfunction, and pronounced renal NO-mediated vasodilation is associated with protection against the development of renal damage in several models of renal injury (11, 20). However, in the present study, no difference or increase (rather than decrease) in NO-mediated vasodilation was found in FHH rats, with no and minor renal damage, respectively, compared with FHL. Increased NO activity, as described in early stages of diabetic nephropathy, may be linked to hyperfiltration (32), and the development of proteinuria, or it may simply represent a compensatory mechanism in response to the production of COX-derived reactive oxygen species. Interestingly, increased constitutive nitric oxide synthase-1 expression has been described in macula densa of young FHH rats (36); however, it is unclear whether NO signaling in pregglomerular arteries is also affected. NO-related changes in FHH rats were not observed in systemic vascular beds, even though ACh-induced relaxation almost entirely relies on NO in the aorta. Taken together, impaired NO-mediated relaxation does not seem to
represent a major mechanism of early vascular dysfunction in spontaneous renal disease.

Endothelial impairment in other vascular beds. In addition to the COX-mediated endothelial dysfunction in small renal arteries (A and B), small mesenteric arteries (C and D), and aorta (E and F). Endothelium-mediated vasodilation to acetylcholine in the absence (total) of any inhibitors and in the presence of indomethacin (indo, 10^-5 mol/l), a combination of indomethacin and N^ω-monomethyl-L-arginine (L-NMMA, 10^-4 mol/l), and eventually in the additional presence of charybdotoxin (chtx, 10^-7 mol/l) and apamin (apa, 3 x 10^-7 mol/l). P < 0.05 indo vs. indo + L-NMMA (*), indo + L-NMMA + chtx + apa vs. all other curves (#), and indo vs. total (>).

Fig. 5. Heterogeneous mechanisms of endothelial dysfunction between 12-wk-old FHL (A, C, and E) and FHH (B, D, and F) rats in small renal arteries (A and B), small mesenteric arteries (C and D), and aorta (E and F). Endothelium-mediated vasodilation to acetylcholine in the absence (total) of any inhibitors and in the presence of indomethacin (indo, 10^-5 mol/l), a combination of indomethacin and N^ω-monomethyl-L-arginine (L-NMMA, 10^-4 mol/l), and eventually in the additional presence of charybdotoxin (chtx, 10^-7 mol/l) and apamin (apa, 3 x 10^-7 mol/l). P < 0.05 indo vs. indo + L-NMMA (*), indo + L-NMMA + chtx + apa vs. all other curves (#), and indo vs. total (>).

Fig. 6. The effect of cyclooxygenase pathway inhibitors on prostanoid-mediated endothelium-dependent contractions in small renal arteries of 12-wk-old FHH rats. The following inhibitors were tested: valeryl salicylate (VAS, 10^-4 mol/l); NS-398 (10^-6 mol/l), a COX-1 selective inhibitor; SQ-29548 (10^-6 mol/l), a COX-2 selective inhibitor; SO-29548 (10^-6 mol/l); COX-2 selective inhibitor; SO-29548 (10^-6 mol/l); COX-2 selective inhibitor; and superoxide dismutase (SOD, 50 U/ml), a thromboxane A2/PGH2 receptor antagonist. *P < 0.05, NS-398 vs. all other inhibitors.

Fig. 5. Heterogeneous mechanisms of endothelial dysfunction between 12-wk-old FHL (A, C, and E) and FHH (B, D, and F) rats in small renal arteries (A and B), small mesenteric arteries (C and D), and aorta (E and F). Endothelium-mediated vasodilation to acetylcholine in the absence (total) of any inhibitors and in the presence of indomethacin (indo, 10^-5 mol/l), a combination of indomethacin and N^ω-monomethyl-L-arginine (L-NMMA, 10^-4 mol/l), and eventually in the additional presence of charybdotoxin (chtx, 10^-7 mol/l) and apamin (apa, 3 x 10^-7 mol/l). P < 0.05 indo vs. indo + L-NMMA (*), indo + L-NMMA + chtx + apa vs. all other curves (#), and indo vs. total (>).

Fig. 6. The effect of cyclooxygenase pathway inhibitors on prostanoid-mediated endothelium-dependent contractions in small renal arteries of 12-wk-old FHH rats. The following inhibitors were tested: valeryl salicylate (VAS, 10^-4 mol/l); NS-398 (10^-6 mol/l), a COX-1 selective inhibitor; SQ-29548 (10^-6 mol/l), a COX-2 selective inhibitor; and superoxide dismutase (SOD, 50 U/ml), a thromboxane A2/PGH2 receptor antagonist. *P < 0.05, NS-398 vs. all other inhibitors.
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DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES