Reduced Reactivity of Renal Microvessels to Pressure and Angiotensin II in Fawn-Hooded Rats

William F. van Rodijnen, Ton A. van Lambalgen, Geert-Jan Tangelder, Richard P.E. van Dokkum, Abraham P. Provoost, Piet M. ter Wee

Abstract—Fawn-Hooded rats possess an increased risk to develop glomerular damage. Both an impaired control of preglomerular resistance and an elevated postglomerular resistance have been implicated. In the present study, we directly assessed the myogenic reactivity of distal interlobular arteries and afferent arterioles from hypertensive and normotensive Fawn-Hooded rats compared with Sprague-Dawley and Wistar rats, which are known to be resistant for developing renal disease. Pressure-response curves were made in isolated perfused hydronephrotic kidneys from these rats. In addition, increasing concentrations of angiotensin II were added to the perfusate to determine the reactivity of interlobular arteries, afferent arterioles, and efferent arterioles to this peptide. Preglomerular vessels from hypertensive and normotensive Fawn-Hooded rats exhibited an impaired reactivity to both pressure and angiotensin II compared with that of Sprague-Dawley and Wistar rats. Basal efferent arteriolar diameters were similar among the 4 strains of rat. In addition, efferent arterioles from hypertensive and normotensive Fawn-Hooded rats displayed a reduced sensitivity to angiotensin II. Our observations demonstrate that in Fawn-Hooded rats, 2 components of preglomerular resistance control are impaired: the myogenic and the angiotensin II response. In addition, efferent arteriolar reactivity to angiotensin II is not elevated but lowered in these rats. Therefore, a deficit in preglomerular resistance control is the most important intrinsic factor involved in the increased susceptibility of Fawn-hooded rats to develop renal disease. (Hypertension. 2002;39:111-115.)

Key Words: rats, Fawn-hooded ■ hydronephrosis ■ renal circulation ■ pressure ■ angiotensin II ■ vasoconstriction

Glomerular hypertension is generally thought to play an important role in the pathophysiology of chronic renal failure.1 In a genetic rat model of this disease, ie, the Fawn-Hooded hypertensive (FHH) rat, an elevated glomerular capillary pressure precedes the development of proteinuria and focal glomerulosclerosis.2,3 In these animals, systemic arterial pressure is already moderately elevated at a relatively young age.4,5 Simons et al6 reported that glomerular capillary pressure correlated closely to the level of systemic arterial pressure in FHH rats treated with antihypertensive agents. Moreover, autoregulation of glomerular capillary pressure was found to be impaired,6 suggesting that a disturbed control of the preglomerular resistance contributes to glomerular hypertension in this strain of rat.

In a genetically closely related normotensive strain of the FHH rat, ie, the Fawn-Hooded low blood pressure (FHL) rat, renal damage develops at a slower rate.7 However, when in these rats systemic arterial blood pressure is elevated by nitro-L-arginine methyl ester treatment, the incidence of focal glomerulosclerosis increases markedly.8 These data indicate that also FHL rats possess an increased susceptibility to develop glomerular hypertension, leading to chronic renal failure eventually. Therefore, we postulate that both FHH and FHL rats possess a deficit in their regulation of preglomerular tone.

Two mechanisms contribute importantly to the regulation of preglomerular tone: the tubuloglomerular feedback mechanism and the myogenic response. Recently, tubuloglomerular feedback responses were found to be intact in Fawn-Hooded (FH) rats.9 Thus, an impaired myogenic reactivity of the preglomerular vessels may underlie the increased susceptibility of FH rats to develop renal injury. So far, only 1 study has addressed this question. Using large proximal interlobular arteries (ILAs), an impaired pressure-induced constriction was found in FHH rats.6 However, other rat strains that are resistant to developing chronic renal failure were not included.
in this study. Moreover, large proximal ILAs are less responsive to pressure rises than are smaller distal ILAs and afferent arterioles (AAs).\textsuperscript{10,11} Consequently, these latter 2 vessel types could have a much greater impact on the total preglomerular resistance during elevated systemic arterial pressure.

Besides a reduced regulation of preglomerular tone, an increased tone of the efferent arterioles (EAs) may also contribute to the increased susceptibility of FH rats to develop renal disease. A high tone of the EAs will retain an elevated glomerular pressure in the capillary network. Previously, Simons et al reported a high postglomerular resistance in FHH rats\textsuperscript{2} and suggested that a preferential action of angiotensin (Ang) II on the EAs could be involved.\textsuperscript{3} At present, however, responsiveness of EAs to Ang II has not been measured directly in FHH and FHL rats.

The first aim of the present study was to determine the myogenic responsiveness of small ILAs and AAs from FHH and FHL rats. Hereto, pressure-response curves were performed in isolated perfused hydronephrotic kidneys. In this preparation, renal autoregulation depends solely on the myogenic mechanism because renal tubules are absent.\textsuperscript{12} For comparison, similar experiments were performed in 2 widely used control strains, ie, Sprague-Dawley (SD) and Wistar rats. Our second aim was to study in these 4 rat strains the reactivity of ILAs, AAs, and EAs to Ang II, with special interest for the sensitivity of the EAs to this peptide.

**Methods**

The in vitro perfused hydronephrotic rat kidney model\textsuperscript{13} was used to determine the renal microvascular reactivity to pressure and Ang II in FHH, FHL, SD, and Wistar rats. All rats were males weighing 225 to 250 g and were housed and handled according to guidelines of the Institutional Animal Care and Use Committee (Vrije Universiteit). Unilateral hydronephrosis was induced by ligating the left ureter under Hypnorm (fentanyl citrate/fluanisone) and diazepam anesthesia. Six to 8 weeks later, tubular tissue atrophy had advanced to a stage that allowed direct visualization of the individual renal microvasculature.\textsuperscript{12,14} At that stage, the rats were 14 to 16 weeks of age, and differences between strains in systolic blood pressure were just starting to develop, being in FHH rats \textsim 140 mm Hg\textsuperscript{2} and in FHL, SD, and Wistar rats between 120 and 125 mm Hg.\textsuperscript{2,15}

To isolate the hydronephrotic kidney, a rat was anesthetized using sodium pentobarbital and intravenously, the renal artery was cannulated via the aorta and perfusion with warm (37°C) Dulbecco’s modified Eagle’s medium (DMEM) was started in vivo. DMEM was slightly modified, containing (in mmol/L) 23.8 sodium bicarbonate, 5.5 D-glucose, 1 sodium pyruvate, and 5.6 HEPES; it was equilibrated with 95% air/5% CO\textsubscript{2}. Under continuous single-pass perfusion, the hydronephrotic kidney was excised and transported to a modified microscope table.\textsuperscript{14} Through a small hole, a light rod was advanced into the kidney, and individual renal microvessels were visualized using transillumination. Images were recorded with a 40\times objective lens on video after at least 1 hour of equilibration. Changes in vessel diameters were assessed using an automated custom designed wall tracking system that measures the distance between dark parallel edges on either site of the vessel wall.\textsuperscript{14} For ILAs, responses were evaluated just before branching distance between dark parallel edges on either site of the vessel wall.\textsuperscript{14} For ILAs, responses were evaluated just before branching.\textsuperscript{14} Myogenic reactivity was determined only for ILAs and AAs. Subsequently, perfusion pressure was returned to 80 mm Hg, and after 30 minutes, responses of ILAs, AAs, and EAs to increasing concentrations of Ang II (3 pmol/L to 1 nmol/L) were assessed.

DMEM, D-glucose and Ang II were obtained from Sigma-Aldrich, sodium bicarbonate was purchased from Merck, and pyruvate and HEPES were from GIBCO-BRL.

All data are presented as mean±SEM. The n value refers to the number of kidneys examined. When multiple vessels were studied in a kidney, the mean value obtained for each vessel type was used. The pressure or Ang II concentration at which half-maximal constriction was observed was calculated. ANOVA followed by a Newman-Keuls post hoc test was performed on the raw data to analyze differences between and within strains. P<0.05 was considered statistically significant. To facilitate comparison between strains, data are depicted in the Figures as the percentage change from basal diameter.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

### Results

Renal microvascular diameters did not differ significantly between the 4 strains of rat (Table 1).

**Renal Microvascular Responses to Pressure**

Figure 1 shows the relative diameter changes of ILAs and AAs to stepwise elevations in renal perfusion pressure in kidneys from the 4 strains of rat. In the FH strains and the control strains, pressure elevation caused a progressive reduction in the diameter of ILAs and AAs. However, for both types of preglomerular vessels, the curves of FHH and FHL rats were shifted to the right of those of SD and Wistar rats, indicating that myogenic responsiveness is reduced in FH rats. In support, maximal pressure-induced constriction of AAs, ie, at 180 mm Hg, tended to be less in kidneys from both FHH and FHL rats (Table 2). Moreover, the threshold pressure required to elicit significant vasoconstriction (see Figure 1) and the pressure at which half of the maximal constriction of ILAs and AAs was observed (Table 3) were clearly higher in the FH strains than in the control strains.

**Renal Microvascular Responses to Ang II**

Diameters of preglomerular vessels had returned to their basal value before Ang II was added to the perfusion medium. Ang II induced a dose-dependent constriction of both pre- and postglomerular vessels (Figure 2). Dose-response curves for ILAs, AAs, and EAs in kidneys from FHH and FHL rats exhibited a rightward shift compared with curves from SD and Wistar rats, indicating that renal microvessels from FH rats are less sensitive to Ang II. In support, the pD\textsubscript{2} values were clearly lower for all tree vessel types in the FH strains than in the control strains (Table 4). In addition, maximal

| TABLE 1. Basal Renal Microvascular Diameters (\textmu m) in Different Strains of Rat |
|-------------------------------|----------------|----------------|
|                               | ILAs           | AAs            |
| FHH                           | 27.6±0.5       | 17.9±0.5       |
| FHL                           | 30.9±1.0       | 18.5±0.3       |
| SD                            | 27.1±1.3       | 18.9±0.7       |
| Wistar                        | 29.6±2.0       | 18.9±0.3       |

<table>
<thead>
<tr>
<th></th>
<th>EAs</th>
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</thead>
<tbody>
<tr>
<td>FHH</td>
<td>17.5±0.5</td>
</tr>
<tr>
<td>FHL</td>
<td>16.4±0.4</td>
</tr>
<tr>
<td>SD</td>
<td>16.8±0.7</td>
</tr>
<tr>
<td>Wistar</td>
<td>17.4±0.6</td>
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</table>

Values are mean±SEM, n=5 to 7.
Ang II–induced vasoconstriction tended to be less in ILAs and AAs from FHH rats and, especially, FHL rats (Table 2).

### Discussion

Both the FHH and FHL rats possess an increased susceptibility to develop chronic renal failure, although in FHH rats signs of this disease, ie, proteinuria and focal glomerulosclerosis, develop more rapidly than in FHL rats. In general, a rise in glomerular capillary pressure is thought to be one of the major risk factors for the development of chronic renal failure. In the present study, we found that in both FH strains, ILAs and AAs exhibited a reduced reactivity to pressure elevation and Ang II compared those in SD and Wistar rats. With regard to postglomerular regulation of glomerular capillary pressure, basal diameters of EAs were similar among the 4 strains of rat. In addition, EAs from FHH and FHL rats displayed a reduced sensitivity to Ang II. Using video-microscopy, the present study is the first to demonstrate directly that distal ILAs and AAs from both FH strains display an impaired constriction to increases in renal perfusion pressure. Because these vessels are known to contribute substantially to renal autoregulation, these findings indicate that in both FH strains, systemic arterial pressure is excessively transmitted into the glomerular capillaries, initiating the development of glomerular damage. In FHH rats, however, proteinuria and glomerulosclerosis develop early in life, whereas in FHL rats signs of glomerular damage do not become overt until these animals are old. Thus, besides the presence of an impaired control of preglomerular resistance, an additional condition has to exist for glomerular damage to progress rapidly. FHH rats differ from FHL rats by their genetic susceptibility to develop systemic hypertension. Moreover, van Dokkum et al reported that the incidence of glomerulosclerosis increased markedly in FHL rats made hypertensive by nitro-L-arginine methyl ester treatment, supporting our hypothesis that both an impaired control of preglomerular

### Table 2. Maximal Renal Microvascular Constriction (Percent Change) to Pressure or Ang II in Different Strains of Rat

<table>
<thead>
<tr>
<th></th>
<th>ILAs</th>
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<th>AAs</th>
<th></th>
<th></th>
<th>EAs</th>
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<tr>
<td></td>
<td>Pressure</td>
<td>Ang II</td>
<td></td>
<td>Pressure</td>
<td>Ang II</td>
<td>Ang II</td>
<td></td>
<td>Ang II</td>
</tr>
<tr>
<td>FHH</td>
<td>-34.5±8.7</td>
<td>-39.2±4.5</td>
<td></td>
<td>-27.7±5.5</td>
<td>-42.0±3.9</td>
<td>-28.0±4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FHL</td>
<td>-40.5±4.9</td>
<td>-36.0±8.0</td>
<td></td>
<td>-25.9±6.2</td>
<td>-35.4±3.3†‡</td>
<td>-32.3±6.7</td>
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<td></td>
</tr>
<tr>
<td>SD</td>
<td>-45.2±3.9</td>
<td>-44.7±4.9</td>
<td></td>
<td>-44.6±4.6</td>
<td>-52.9±3.8</td>
<td>-27.1±5.3</td>
<td></td>
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</tr>
<tr>
<td>Wistar</td>
<td>-43.9±4.3</td>
<td>-47.4±3.3</td>
<td></td>
<td>-39.7±3.5</td>
<td>-50.1±3.4</td>
<td>-31.8±2.0</td>
<td></td>
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</tbody>
</table>

Values are mean±SEM, n=5 to 7. P<0.05 vs Wistar, SD, and FHH rats.

### Table 3. Sensitivity (in mm Hg) of Preglomerular Vessels to Increases in Renal Perfusion Pressure in Different Strains of Rat

<table>
<thead>
<tr>
<th></th>
<th>ILAs</th>
<th>AAs</th>
</tr>
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<tbody>
<tr>
<td>FHH</td>
<td>131±11*</td>
<td>119±6</td>
</tr>
<tr>
<td>FHL</td>
<td>124±7</td>
<td>122±5*</td>
</tr>
<tr>
<td>SD</td>
<td>108±4</td>
<td>111±3</td>
</tr>
<tr>
<td>Wistar</td>
<td>100±4</td>
<td>103±5</td>
</tr>
</tbody>
</table>

Values are mean±SEM and used as an inverse of sensitivity, ie, the lower the value the higher the sensitivity for pressure.

*P<0.05 vs Wistar rats, n=6 or 7.
resistance and an elevated systemic arterial pressure are needed for renal disease to progress rapidly. In this study, experiments were performed using hydronephrotic rat kidneys, which are devoid of the tubuloglomerular feedback mechanism. Thus, the impaired pressure-induced constriction of ILAs and AAs in kidneys from FHH and FHL rats reflects an impaired myogenic reactivity of these microvessels. This finding is in agreement with previous conclusions drawn from blood flow measurements using Laser-Doppler flowmetry. For example, autoregulation of renal medullary and cortical blood flow was found to be impaired in FHH rats that were volume-loaded to inhibit tubuloglomerular feedback responses. In addition, micropuncture measurements showed an intact tubuloglomerular feedback mechanism in kidneys from FH rats, also suggesting an impaired myogenic response as the cause of a disturbed renal autoregulation in FH rats. Recent experiments using cannulated proximal ILAs with a diameter of ≈100 μm demonstrated an impaired pressure-induced constriction of these vessels in FHH rats. These findings in combination with our results indicate that in FHH rats, myogenic reactivity is impaired throughout the entire preglomerular vascular tree. In FHL rats, however, proximal ILAs still constricted in the pressure range from 70 to 120 mm Hg, which is an additional difference between FHH and FHL rats, making FHL rats less susceptible to develop renal damage.

It is interesting to note that the reduced myogenic reactivity of preglomerular arterioles in FH rats is paralleled by a reduced reactivity to Ang II. This correlation becomes most clear when data from Table 3 are compared with those from Table 4, showing that ILAs and AAs from FHH and FHL rats possess a lower sensitivity to both pressure and Ang II than did the 2 control strains. At present, it remains to be resolved why preglomerular vessels from FH rats are less sensitive to these 2 vasoconstrictive stimuli. Our finding that vasoconstriction in FH rats is impaired to both pressure and Ang II suggests that these rats possess a disturbance in a common part of intracellular pathway leading to smooth muscle cell contraction. Studies directed at, eg, the expression or activity of second messengers or ion channels in smooth muscle cells from preglomerular vessels of FH rats might give further insight in the molecular mechanism involved.

Potentially, an elevated resistance of the EAs could also increase the risk to develop renal disease. Previously, Simons et al calculated postglomerular resistance in different strains of rat and reported a higher value in FHH rats than in FHL and Wistar rats. However, our direct observations did not indicate an increase in efferent arteriolar tone in kidneys from FH rats. Basal diameters of EAs were similar among the 4 strains of rat, suggesting that in EAs from FH rats, structural changes are absent. In addition, both the preglomerular vessels and the EAs from FH rats constricted to Ang II, making it less likely that a preferential action of Ang II on the EAs would be responsible for an increased glomerular capillary pressure. On the contrary, we found that EAs from FH rats displayed a reduced sensitivity to Ang II, suggesting that

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**Figure 2.** Ang II–induced diameter changes of ILAs (upper panel), AAs (middle panel), and EAs (lower panel) in kidneys from the 4 strains of rat. Values are mean±SEM, n=5 to 7. *P<0.05 vs basal diameter for this point and the following part of the dose-response curve.

**Table 4. Sensitivity of Renal Microvessels to Angiotensin II in Different Strains of Rat**

<table>
<thead>
<tr>
<th></th>
<th>ILAs</th>
<th>AAs</th>
<th>EAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHH</td>
<td>10.12±0.21</td>
<td>10.20±0.13</td>
<td>10.09±0.21*†</td>
</tr>
<tr>
<td>FHL</td>
<td>9.73±0.16*†</td>
<td>9.97±0.12*</td>
<td>10.07±0.09*†</td>
</tr>
<tr>
<td>SD</td>
<td>10.47±0.22</td>
<td>10.37±0.25</td>
<td>10.78±0.20</td>
</tr>
<tr>
<td>Wistar</td>
<td>10.71±0.07</td>
<td>10.68±0.12</td>
<td>10.71±0.08</td>
</tr>
</tbody>
</table>

Data are depicted as the −log of the concentration Ang II that elicited 50% of the maximal constriction (pD2-value). Values are mean±SEM, n=5 to 7. P<0.05 vs *Wistar and †SD rats.
Efferent arteriolar resistance is lower in FH rats compared with control rats.

In conclusion, the present study demonstrates that the ability of ILAs and especially AAs to respond to an increase in renal perfusion pressure and to Ang II is reduced in FH rats. Consequently, changes in blood pressure are less well buffered, and systemic pressure is excessively transmitted to the glomerulus. Because of this, glomerular hypertension occurs more easily, making these animals more susceptible to the development and progression of chronic renal failure.

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References
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