Renal Arteriolar Diameters in Spontaneously Hypertensive Rats
Vascular Cast Study

Kenjiro Kimura, Akihiro Tojo, Hiroaki Matsuoka, and Tsuneaki Sugimoto

The relation between the arteriolar diameters and hypertensive glomerulosclerosis was studied by using microvascular casts and histological evaluation. Spontaneously hypertensive rats 4 weeks of age were divided into three groups: nontreated, captopril (40 mg/kg/day)-treated, and trichlormethiazide (1 mg/kg/day) with hydralazine (20 mg/kg/day)-treated. Wistar-Kyoto rats served as controls. At 6 weeks old, the captopril-treated rats showed a lower blood pressure and a larger afferent arteriolar diameter compared with the control rats. At 20 weeks old, the nontreated group exhibited hypertension and a lower arteriolar diameter ratio (afferent to efferent, 0.89 versus 1.22 in control group) because of afferent constriction and efferent dilatation, seen equally in the outer and inner cortices. Glomerulosclerosis was accentuated only in the inner cortex of the nontreated group (score, 63 versus 29 in control group). In the two treated rat groups, the blood pressure was reduced and arteriolar diameter ratios were similar to those in the control group (1.18 and 1.26). The sclerosis score in the trichlormethiazide with hydralazine-treated rats (score, 26) was lower than in the nontreated rats but not the captopril-treated rats (score, 36). These results indicated that 1) in the hypertensive rats, despite a reduced diameter ratio, glomerulosclerosis was more severe in the inner cortex; 2) two therapies reduced blood pressure and reversed the arteriolar changes, but a decrease in glomerulosclerosis was seen only in the trichlormethiazide with hydralazine-treated rats; and 3) for development of glomerulosclerosis, factors other than hemodynamics may be important in addition to intraglomerular pressure. (Hypertension 1991;18:101-110)

Recent micropuncture studies have shown a close relation between elevation in glomerular pressure and glomerular damage in various experimental renal diseases, including hypertension.1-5 Because the preglomerular and postglomerular vascular resistance is one of the major factors for regulation of glomerular pressure,6 quantitative evaluation of changes in the arteriolar diameter would facilitate our understanding about the pathogenesis of hypertensive glomerular damage. Direct and precise measurement of the arteriolar diameter has been achieved by several different techniques (i.e., isolated arterioles,7 the hydronephrosis model,8 the juxtamedullary nephron model,9 and the vascular cast using resin10-14). Among them, however, only vascular casts facilitate evaluation of arterioles in normally functioning intact kidneys in situ.10-14 Earlier microvascular cast studies have demonstrated that the afferent arterioles in spontaneously hypertensive rats (SHR) are smaller than those in normotensive control Wistar-Kyoto (WKY) rats.10-12 However, because the efferent arterioles are as important as the afferent arterioles in determination of the glomerular pressure,6,15 we recently have examined the efferent arterioles simultaneously with the afferent arterioles by using the same method and have reported that the efferent arterioles are dilated, whereas the afferent arterioles are constricted in the adult SHR.16

There were two purposes in the present cast study: first, to evaluate the effects of antihypertensive therapies on renal arterioles and glomerulosclerosis in SHR; and second, to reveal regional differences in arteriolar changes, because the vascular casts facilitate separate observation of the outer and inner cortices.

**Methods**

**Experimental Design**

Thirty 4-week-old male SHR and 11 normotensive control male WKY rats of the same age (WKY group) were used. The SHR were assigned at random to three groups: captopril (40 mg/kg body wt/day)-
treated (SHR+CAP group, \(n=10\)), trichlormethiazide (1 mg/kg body wt/day) and hydralazine (20 mg/kg body wt/day)-treated (SHR+TH group, \(n=9\)), and nontreated (SHR group, \(n=11\)). These antihypertensive drugs were given to the rats by mixing with normal rat chow. Before the experiments, the amount of chow taken by a rat was examined and found to average 100 g/kg body wt/day. Based on this observation, the antihypertensive drugs were mixed with rat chow so that the expected amounts of drugs described above were taken by rats. The amount of chow consumed by the animals was checked every week during the experiment and was found to be comparable among the four experimental groups. Separately from the experiments, plasma captopril concentration was measured with high-performance liquid chromatography in five normal Wistar rats after 7 days of administration of the captopril-containing chow, which was the same as that given to the SHR+CAP group. The plasma concentration of captopril varied from 320 to 560 (mean 420) ng/ml, and this plasma level was considered to reach the effective pharmacological level.

The body weight and blood pressure were measured three times during the experiment: before drug treatment (at 4 weeks old), after 2 weeks of administration of the drugs (at 6 weeks old), and finally after 16 weeks of administration of the drugs (at 20 weeks old). Systolic blood pressure was measured by the tail-cuff method with an electric manometer (Nihon Koden Co., Tokyo) on conscious rats, and three measurements were averaged.

Renal microvascular cast and histological studies were performed at 6 weeks old using five rats from each group except the SHR+TH group (\(n=4\)) and at 20 weeks old using the remaining rats from each group.

### Microvascular Cast Study

Microvascular casts were prepared following the method of Gattone et al. Under pentobarbital anesthesia, a polyethylene catheter (PE-60) was inserted retrogradely into the abdominal aorta, and the systemic blood pressure was monitored via the catheter by using a three-way stopcock, which was connected to an electric manometer (Natsume Co., Tokyo) on conscious rats, and histological examination and transferred to a sputter coater (Eiko Co., Kanagawa, Japan) for coating with gold palladium for 90 seconds at 1.0 mV. A scanning electron microscope (S-450, Hitachi, Ibaraki, Japan) for examining and photographing the casts. The glomerular casts were subsequently dissected under a stereomicroscope (Nikon Co., Tokyo). Casting was performed up to efferent arterioles in some nephrons (about 30%) and only up to glomeruli in the rest. Casts were fragile and easily destroyed during the procedure; therefore, we decided on three criteria for choosing casts. First, glomerular casts should preserve afferent arterioles connected to the interlobular arteries so that we could identify the cortical region to which glomeruli belonged. Second, glomerular casts should also accompany the efferent arterioles so that we could evaluate the relation between the afferent and efferent arterioles; efferent arteriolar casts varied from 10 to 150 \(\mu m\) in length. Efferent arteriolar casts more than 50 \(\mu m\) in length were chosen to evaluate the variations in diameter. Finally, glomerular casts should not have major defects in capillary tufts; therefore, sclerotic glomeruli were excluded from the evaluation because once glomeruli become sclerotic, the glomerular hemodynamics and thus the arteriolar diameters are probably greatly altered. We assumed that only nonsclerotic glomeruli should be evaluated to clarify the relation of arterioles to the development of hypertensive glomerular damage. Three to six glomeruli that fulfilled the above criteria were examined in each outer cortex, and the same number of glomeruli in each inner cortex in one kidney. The total number of glomerular casts examined in each cortical region in each group totaled 25-35.

To differentiate outer cortical glomeruli from inner cortical glomeruli, interlobular arteries were arbitrarily divided into upper and lower parts. Glomeruli whose afferent arterioles came from the upper parts of interlobular arteries were defined as outer cortical glomeruli, and glomeruli whose afferent arterioles came from the lower parts of interlobular arteries were defined as inner cortical glomeruli. Juxtamedullary glomeruli from which afferent arterioles originated directly from arcuate arteries were included in the inner cortical glomeruli. The casts were mounted on stubs for scanning electron microscopic examination and transferred to a sputter coater (Eiko Co., Kanagawa, Japan) for coating with gold palladium for 90 seconds at 1.0 mV. A scanning electron microscope (S-450, Hitachi, Japan) was used for examining and photographing the casts.

Casts were examined in the scanning electron microscope at 600x, using an accelerating voltage of 20 kV and a working distance of 15 mm. The diameters of arterioles were estimated on the photomicrographs as previously described. In a segment of arteriole 50 \(\mu m\) from the glomerulus, the
Statistical Analysis

The measured data were expressed as mean±SEM.

Body weight, blood pressure, and arteriolar diameter. The data from four groups were first submitted to one-way analysis of variance, and when a probability value obtained was less than 0.05, multiple t tests were performed on paired groups using the Bonferroni method,20 for which a value of p<0.004 was required for statistical significance. Arteriolar diameters and their ratios from the outer cortex were compared with those from the inner cortex (the regional differences) based on Student's t test. Diameters of afferent arterioles without corresponding efferent arterioles were compared with diameters of afferent arterioles with corresponding efferent arterioles using Student's t test. In Student's t test, a value of p<0.05 was considered significant.

Glomerulosclerosis score. Because the distribution is not necessarily normal, nonparametric tests were applied. The data from four groups were first analyzed by using the Kruskal-Wallis H test, and when a probability value was less than 0.05, a Wilcoxon rank sum test was applied to paired groups, for which a value of p<0.05 was required for statistical significance.

Results

Body Weight and Blood Pressure

The body weights (Figure 1) of the four groups were similar at 4 weeks of age before the experiment started (98±2 g in WKY, 101±3 g in SHR, 100±3 g in SHR+CAP, and 99±3 g in SHR+TH). At 6 weeks old, however, the body weights of the two treated SHR groups were significantly lower than those of the SHR group (110±5 g in SHR+CAP and 103±5 g in SHR+TH versus 134±4 g in SHR, both p<0.0008; WKY, 121±6 g). The body weights of the four groups were similar again at age 20 weeks (348±13 g in WKY, 342±12 g in SHR, 308±30 g in SHR+CAP, and 381±24 g in SHR+TH).

The systolic blood pressures (Figure 2) of the four groups were similar at 4 weeks old (116±7 mm Hg in WKY, 124±1 mm Hg in SHR, 122±2 mm Hg in SHR+CAP, and 124±1 mm Hg in SHR+TH). At 6 weeks old, the difference between the blood pressures of the SHR and the WKY groups was not significant (129±5 mm Hg in SHR versus 117±5 mm Hg in WKY, NS). The blood pressures of the two treated SHR groups were significantly lower than those of the SHR group (97±4 mm Hg in SHR+CAP and 104±2 mm Hg in SHR+TH, both p<0.0008 versus SHR). The blood pressure of the SHR+CAP group was also significantly lower than that of the WKY group (p<0.004). At 20 weeks old, the blood pressure of the SHR group was markedly higher than that of the WKY group (197±10 mm Hg in SHR versus 156±7 mm Hg in WKY, p<0.004). The blood pressures of the two treated SHR groups were significantly lower than that of the SHR group (126±6 mm Hg in SHR+CAP and 149±3 mm Hg in SHR+TH, both p<0.0008 versus SHR) and comparable with that of the WKY group. The blood pressure of the SHR+CAP group was lower than that of the SHR+TH group (p<0.004).

Renal Arteriolar Diameters

The data are shown for the whole cortex, unless otherwise indicated (Figure 3).

Six weeks old. The afferent arteriolar diameter of the SHR group was comparable with that of the WKY group (11.4±0.4 μm in SHR versus 11.6±0.6 μm in WKY, NS) (Figure 3A). However, the afferent...
arteriolar diameter of the SHR+CAP group was markedly larger than those of the other three groups (15.1±0.7 µm, p<0.004 versus WKY, p<0.0008 versus SHR and SHR+TH). The afferent arteriolar diameter of the SHR+TH group was similar to those of the WKY and the SHR groups (10.7±0.7 µm). The efferent arteriolar diameters of the four groups were similar at this stage (9.8±0.6 µm in WKY, 10.6±0.6 µm in SHR, 9.4±0.4 µm in SHR+CAP and 8.4±0.5 µm in SHR+TH).

The ratio of the afferent to efferent arteriolar diameters (a/e ratio) in the SHR+CAP was significantly larger than those of the other three groups (1.64±0.05 in SHR+CAP versus 1.20±0.03 in WKY, 1.10±0.04 in SHR, and 1.31±0.07 in SHR+TH; all p<0.0008) (Figure 3B). The a/e ratios of the WKY, the SHR, and the SHR+TH groups were comparable.

Twenty weeks old. Representative arteriolar casts from the WKY, the SHR, and the SHR+CAP groups at this stage are shown in Figure 4.

The afferent arteriolar diameter of the SHR group was significantly smaller than those of the other three groups (12.3±0.5 µm in SHR versus 14.0±0.5 µm in WKY, p<0.004; 15.5±0.3 µm in SHR+CAP, p<0.0008; and 15.4±0.3 in SHR+TH, p<0.0008) (Figure 3C). The afferent arteriolar diameters of the WKY, the SHR+CAP, and the SHR+TH groups were comparable. The efferent arteriolar diameter of the SHR was significantly larger than that of the WKY group (14.0±0.5 µm in SHR versus 11.9±0.4 µm in WKY, p<0.0008).

**FIGURE 1.** Bar graphs show body weight at age 4 weeks (before drug treatment), 6 weeks (2 weeks treatment), and 20 weeks (16 weeks treatment). WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; SHR+CAP, SHR treated with captopril (40 mg/kg body wt/day); SHR+TH, SHR treated with trichlormethiazide (1 mg/kg body wt/day) and hydralazine (20 mg/kg body wt/day). *p<0.004, **p<0.0008 (Bonferroni method).

**FIGURE 2.** Blood pressure at age 4 weeks (before drug treatment), 6 weeks (2 weeks treatment), and 20 weeks (16 weeks treatment). WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; SHR+CAP, SHR treated with captopril (40 mg/kg body wt/day); SHR+TH, SHR treated with trichlormethiazide (1 mg/kg body wt/day) and hydralazine (20 mg/kg body wt/day). *p<0.004, **p<0.0008 (Bonferroni method).
arteriolar diameter of the SHR+CAP group was markedly larger than those of the other three groups (15.1±0.7 μm, p<0.004 versus WKY, p<0.0008 versus SHR and SHR+TH). The afferent arteriolar diameter of the SHR+TH group was similar to those of the WKY and the SHR groups (10.7±0.7 μm). The efferent arteriolar diameters of the four groups were similar at this stage (10.6±0.6 μm in SHR and 9.4±0.4 μm in SHR+CAP and 8.4±0.5 μm in SHR+TH).

The ratio of the afferent to efferent arteriolar diameters (a/e ratio) in the SHR+CAP was significantly larger than those of the other three groups (1.64±0.05 in SHR+CAP versus 1.20±0.03 in WKY, 1.10±0.04 in SHR, and 1.31±0.07 in SHR+TH; all p<0.0008) (Figure 3B). The a/e ratios of the WKY, the SHR, and the SHR+TH groups were comparable.

Twenty weeks old. Representative arteriolar casts from the WKY, the SHR, and the SHR+CAP groups at this stage are shown in Figure 4.

The afferent arteriolar diameter of the SHR group was significantly smaller than those of the other three groups (12.3±0.5 μm in SHR versus 14.0±0.5 μm in WKY, p<0.004; 15.5±0.3 μm in SHR+CAP, p<0.0008; and 15.4±0.3 in SHR+TH, p<0.0008) (Figure 3C). The afferent arteriolar diameters of the WKY, the SHR+CAP, and the SHR+TH groups were comparable. The efferent arteriolar diameter of the SHR was significantly larger than that of the WKY group (14.0±0.5 μm in SHR versus 11.9±0.4 μm in WKY, p<0.0008).

**Figure 1.** Bar graphs show body weight at age 4 weeks (before drug treatment), 6 weeks (2 weeks treatment), and 20 weeks (16 weeks treatment). WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; SHR+CAP, SHR treated with captopril (40 mg/kg body wt/day); SHR+TH, SHR treated with trichlormethiazide (1 mg/kg body wt/day) and hydralazine (20 mg/kg body wt/day). **p<0.0008 (multiple t test using the Bonferroni method).**

**Figure 2.** Blood pressure at age 4 weeks (before drug treatment), 6 weeks (2 weeks treatment), and 20 weeks (16 weeks treatment). WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; SHR+CAP, SHR treated with captopril (40 mg/kg body wt/day); SHR+TH, SHR treated with trichlormethiazide (1 mg/kg body wt/day) and hydralazine (20 mg/kg body wt/day). *p<0.004, **p<0.0008 (Bonferroni method).
Table 1. Arteriolar Diameters in the Outer and Inner Cortices

<table>
<thead>
<tr>
<th>Category</th>
<th>WKY</th>
<th>SHR</th>
<th>SHR+CAP</th>
<th>SHR+TH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>6 weeks old</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outer cortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Afferent</td>
<td>11.4±0.7</td>
<td>11.1±0.7</td>
<td>14.9±1.0*</td>
<td>10.3±0.7</td>
</tr>
<tr>
<td>Efferent</td>
<td>9.5±0.5</td>
<td>10.9±0.9</td>
<td>8.9±0.5</td>
<td>8.1±0.5</td>
</tr>
<tr>
<td>a/ef</td>
<td>1.16±0.05</td>
<td>1.02±0.04</td>
<td>1.72±0.05†</td>
<td>1.25±0.07‡</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Inner cortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Afferent</td>
<td>11.8±0.6</td>
<td>11.7±0.8</td>
<td>15.2±1.2*</td>
<td>11.1±0.7</td>
</tr>
<tr>
<td>Efferent</td>
<td>10.1±0.7</td>
<td>10.2±0.4</td>
<td>9.8±0.6</td>
<td>8.6±0.6</td>
</tr>
<tr>
<td>a/ef</td>
<td>1.18±0.04</td>
<td>1.12±0.06</td>
<td>1.52±0.11§</td>
<td>1.28±0.10</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td><strong>20 weeks old</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outer cortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Afferent</td>
<td>14.2±0.3</td>
<td>12.7±0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Efferent</td>
<td>12.1±0.6</td>
<td>13.3±0.6</td>
<td></td>
<td>#</td>
</tr>
<tr>
<td>a/ef</td>
<td>1.27±0.12#</td>
<td>0.92±0.10**</td>
<td>1.18±0.09§</td>
<td>1.16±0.08</td>
</tr>
<tr>
<td>“Afferent”</td>
<td>14.6±0.7</td>
<td>12.3±0.9††</td>
<td>14.5±0.5</td>
<td>15.0±0.6</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Inner cortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Afferent</td>
<td>13.8±0.8</td>
<td>11.7±0.8††</td>
<td>15.4±0.3</td>
<td>15.1±1.0</td>
</tr>
<tr>
<td>Efferent</td>
<td>12.8±1.3</td>
<td>14.7±0.6#</td>
<td>14.1±0.8</td>
<td>11.5±0.3</td>
</tr>
<tr>
<td>a/ef</td>
<td>1.08±0.05#</td>
<td>0.77±0.06‡‡</td>
<td>1.05±0.04‡</td>
<td>1.25±0.05</td>
</tr>
<tr>
<td>“Afferent”</td>
<td>14.5±0.8</td>
<td>12.1±0.6††</td>
<td>14.9±0.7</td>
<td>14.7±1.0</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

SHR, nontreated spontaneously hypertensive rats; SHR+CAP, captopril (40 mg/kg/day)-treated rats; SHR+TH, trichlormethiazide (1 mg/kg/day) and hydralazine (20 mg/kg/day)-treated rats; afferent, afferent arteriolar diameters (μm); efferent, efferent arteriolar diameters (μm); a/ef, ratio of afferent to efferent arteriolar diameters; n, number of rats examined in each group that were used for statistical evaluation among groups; “Afferent,” diameters of afferent arteriole without corresponding efferent arteriole. Analysis of outer cortex vs. inner cortex was by Student’s t test; all other analyses were by Bonferroni method.

*p<0.004 and †p<0.0008 vs. WKY, SHR, and SHR+TH.

The separate data from outer and inner cortices and the regional differences. The data from the outer and inner cortices are shown separately in Table 1.

At 6 weeks old, in the outer and inner cortices, the afferent arteriolar diameter and the a/ef ratio were largest in the SHR+CAP group. At 20 weeks old, the afferent arteriole of the SHR group was smaller than that of other groups in the outer and inner cortices. The efferent arteriolar diameter of the SHR in the outer cortex was larger than that of the WKY group. The a/ef ratios in the outer and inner cortices were smaller in the SHR than in the WKY group (p<0.004 in the outer cortex, p<0.0008 in the inner cortex). These separate data in the outer and inner cortices were almost agreeable with the combined data shown in Figure 3.

The regional difference was found only at age 20 weeks. In the SHR group, the efferent arteriolar diameter in the inner cortex was significantly larger than that in the outer cortex. The a/ef ratio was significantly smaller in the inner cortex of the WKY and SHR+CAP groups than in the outer cortex of the corresponding groups.

Diameters of afferent arterioles without corresponding efferent arterioles. The afferent arterioles that had no corresponding efferent arterioles were studied at 20 weeks old (shown as “afferent”) (Table 1). They showed diameters similar to the afferent arterioles with corresponding efferent arterioles in each group; no statistical difference was found (Student’s t test). Furthermore, in the outer cortex and in the inner cortex, the afferent arteriolar diameter was smallest in the SHR group (p<0.004 versus WKY, SHR+CAP, and SHR+TH). Thus, the results ob-
tained in these afferent arterioles were quite comparable with those obtained in the afferent arterioles with corresponding efferent arterioles.

**Glomerulosclerosis**

The data are shown for the whole cortex, unless otherwise indicated (Figure 5).

**Six weeks old.** At this stage, any significant difference in the sclerosis score was found among the four groups (29 ±6 in SHR versus 20 ±6 in WKY, 22 ±7 in SHR+CAP, and 16 ±2 in SHR+TH, NS, the Kruskal-Wallis H test) (Figure 5A).

**Twenty weeks old.** The sclerosis score of the SHR group was markedly elevated and significantly higher than those of the WKY and the SHR+TH groups (47±4 in SHR versus 29±4 in WKY, and 26±2 in SHR+TH, both p<0.01, the Wilcoxon test) (Figure 5B). The difference between the score in the SHR and the score in the SHR+CAP groups (36 ±6) was not significant.

<table>
<thead>
<tr>
<th>Category</th>
<th>WKY</th>
<th>SHR</th>
<th>SHR+CAP</th>
<th>SHR+TH</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 weeks old</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outer cortex</td>
<td>19±8</td>
<td>29±8</td>
<td>18±4</td>
<td>18±1</td>
</tr>
<tr>
<td>Inner cortex</td>
<td>22±4</td>
<td>30±5</td>
<td>26±10</td>
<td>15±2</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

| 20 weeks old |
| Outer cortex | 28±4 | 36±2* | 37±6 | 25±3 |
| Inner cortex | 29±5 | 63±10† | 37±7‡ | 26±2 |
| n | 6 | 6 | 5 | 5 |

SHR, nontreated spontaneously hypertensive rats; SHR+CAP, captopril (40 mg/kg/day)-treated rats; SHR+TH, trichlormethiazide (1 mg/kg/day) and hydralazine (20 mg/kg/day)-treated rats.

* p<0.05 the outer cortex vs. the inner cortex (the regional difference).
† p<0.01 vs. WKY and SHR+TH.
‡ p<0.05 vs. SHR+TH.

At age 6 weeks, no significant difference was observed among the four groups, in either the outer cortex or the inner cortex (Table 2), which was consistent with the overall data shown in Figure 5.

At age 20 weeks, neither significant difference was found in the outer cortex among the four groups. However, in the inner cortex, the glomerulosclerosis score of the SHR group was significantly higher than the sclerosis scores of the WKY and SHR+TH groups (both, p<0.01). The score in the SHR+TH group was significantly lower than in the SHR+CAP group (p<0.05) in the inner cortex.

The score of the SHR was remarkably higher in the inner cortex than in the outer cortex at age 20 weeks (p<0.05). Otherwise, no significant regional difference was found.

**Discussion**

In the present study, we confirmed the results of our previous study that the afferent arterioles are constricted and the efferent arterioles are dilated in 20-week-old SHR when compared with WKY rats, and we showed that antihypertensive therapy abolishes the afferent arteriolar constriction, normalizes the arteriolar diameter ratio, and reduces glomerulosclerosis.

Renal vascular casts have been applied in some experimental conditions and have been shown to simulate physiological vascular conditions when perfusion fixation is performed before casting at controlled pressure. Intravascular perfusion fixation with glutaraldehyde prevents vasoconstriction and preserves the vessels in their functional state. In the previous study, we found that changes in arteriolar diameters measured by using vascular casts were well correlated with changes in renal function during the atrial natriuretic peptide infusion. In the present study, we therefore used vascular casts in the quan-
titative assessment of renal arterioles of SHR and in intervention studies using antihypertensive drugs.

To validate simultaneous evaluation of the afferent and efferent arterioles in the cast study, it was necessary to exclude the possibility that the relatively few afferent arterioles accompanied by corresponding efferent arterioles did not represent whole afferent arterioles in the kidney. As seen in Table 1, the results obtained in the afferent arterioles without the efferent arteriole (shown as “afferent”) were quite comparable with those obtained in the afferent arterioles with corresponding efferent arterioles. This fact indicates that the afferent arterioles used in the present simultaneous evaluation with the corresponding efferent arterioles represented the general population of the afferent arterioles, although the number of them in the whole casts was small.

At 6 weeks old, outstanding features were found in the SHR+CAP group: blood pressure reduction and afferent arteriolar dilatation. In the SHR, an elevation in the blood pressure was not significant, and arteriolar diameters were comparable with those in the WKY rats. The blood pressure in the two treated SHR groups was significantly lower than that in the SHR group. Only the SHR+CAP group, however, showed significantly lower blood pressure than the WKY group. The afferent arteriolar diameter and the a/e ratio of the SHR+CAP group were significantly larger than those in the other three groups. It has been shown that the preglobular arteriole plays an important role in maintaining the glomerular pressure (autoregulation). When the perfusion pressure falls, preglobular arteriolar resistance decreases and vice versa so that the glomerular pressure remains constant. Thus the arteriolar dilatation seen in the SHR+CAP group might be attributed to the autoregulation in response to perfusion pressure reduction. However, direct effects of captopril on the afferent arteriole was not totally excluded (mentioned later). At this stage, there was no significant difference in the efferent arteriolar diameters among the four groups. Neither was there a significant difference in the glomerulosclerosis among the four groups.

At 20 weeks old, outstanding features were seen in the SHR: blood pressure elevation, arteriolar diameter changes, and accentuated glomerulosclerosis. In the SHR, the afferent arteriolar diameter was smaller and the efferent arteriolar diameter larger compared with those in the WKY rats; thus the a/e ratio was decreased. Earlier microvascular cast studies by other researchers have shown afferent arteriolar constriction in SHR. Micropuncture studies support these findings, showing elevation in the afferent arteriolar vascular resistance in SHR. The autoregulation of the glomerular filtration has been proposed for the afferent arteriolar constriction. Robertson et al showed by the micropuncture technique that an efferent arteriolar resistance tends to decrease slightly when the systemic pressure rises in normal Wistar rats. Efferent arteriolar dilatation in SHR was shown morphologically for the first time in our previous study and was confirmed in the present study. We recently observed afferent arteriolar constriction and efferent arteriolar dilatation similarly in a different model of hypertension, deoxycorticosterone-salt hypertensive rats. However, the precise mechanisms of these arteriolar changes in hypertension are a subject for future study.

An elevation in the glomerular pressure has been considered to be one of the main factors for the progression of glomerulosclerosis in the SHR. Therefore, arteriolar changes seen in the SHR are assumed to contribute to the protection of a glomerulus from the development of sclerosis through the intraglomerular pressure reduction. The glomerulosclerosis was, however, found to be significantly enhanced in the SHR at age 20 weeks. This inconsistency is discussed later in relation to the regional difference.

At age 20 weeks, two different antihypertensive therapies were effective in reducing the blood pressure; thus, there was no significant difference in blood pressures among the WKY, the SHR+CAP, and the SHR+TH groups. The afferent and efferent arteriolar diameters and the a/e ratio were also comparable among the WKY, the SHR+CAP, and the SHR+TH groups. Similarly, there was no significant difference in the glomerulosclerosis among the WKY, the SHR+CAP, and the SHR+TH groups, which might be attributed to reduction in the glomerular pressure through the systemic pressure reduction. These intervention studies clearly show that arteriolar changes and enhanced glomerulosclerosis in SHR are reversible by reducing the systemic pressure.

When compared between two treated groups, an inconsistency was seen between reduction in the blood pressure and attenuation in the glomerulosclerosis. The blood pressure in the SHR+CAP group was significantly lower than in the SHR+TH group (p<0.004, the Bonferroni method). Although the glomerulosclerosis score in the SHR+TH group was significantly lower than in the SHR, the difference between the score in the SHR and the score in the SHR+CAP groups was not significant. Thus, although captopril was more effective in reducing the blood pressure of the SHR than was the combined therapy of trichlomethiazide and hydralazine, the latter was more effective in reducing glomerulosclerosis. Because we did not evaluate arteries proximal to the afferent arteriole, it is a possible hypothesis that different effects of the two therapies on arteries proximal to the afferent arterioles might cause the different effect on the progression of glomerulosclerosis (i.e., if captopril dilated the interlobular arteries more effectively than the combined therapy, the prevention of glomerulosclerosis by captopril is less effective because of blunting glomerular pressure reduction). Dworkin et al suggested the possibility that captopril produces abnormalities of cortical vessels via a mechanism not dependent on the systemic...
hypertension. This raises an alternative hypothesis that such captopril-induced vascular abnormalities might interfere with the prevention of glomerulosclerosis. Such captopril effect on arteries or arterioles might be related with the prominent afferent arteriolar dilatation seen in the 6-week-old SHR. However, at present we have no definite explanation for this discrepancy; thus, further study, including more systematic investigation of renal vasculature, is necessary to solve this problem.

In the present study, the arteriolar diameters and glomerulosclerosis were examined separately in the outer and inner cortexes, and regional differences were studied. There was no qualitative difference between the arteriolar changes in the outer and inner cortexes at either 6 weeks or 20 weeks of age, and these separate data of the arteriolar changes were consistent with the data from the overall cortex shown in Figure 3. Concerning the regional difference in arteriolar diameters, an earlier microvascular cast study reported that the efferent arterioles in the inner cortex are larger than in the outer cortex. In the present study, the efferent arterioles in the inner cortex were larger than those in the outer cortex in the SHR at 20 weeks old. In the WKY and the SHR+CAP groups, the a/e ratios were significantly smaller in the inner cortex than in the outer cortex, which indicates larger efferent arterioles in the inner cortex. In the 20-week-old SHR, the glomerulosclerosis was significantly higher in the inner cortex than in the outer cortex. The glomerulosclerosis score in the outer cortex was comparable among the four groups. Thus, the accentuated glomerulosclerosis of the SHR shown in the overall data (Figure 5) is attributed exclusively to the accentuated sclerosis in the inner cortex.

Applying the micropuncture technique to SHR, Bank et al. showed that outer cortical glomeruli have normal hydraulic pressure because of elevation of the afferent arteriolar resistance and that the inner cortical glomeruli show high glomerular filtration rate, presumably associated with excessively high glomerular pressure or blood flow. They proposed an intrinsic vascular abnormality in the blood vessels supplying the inner glomeruli as the cause of hyperfiltration. Olsen et al. also suggested breakdown in control of glomerular hemodynamics in the deep cortex in SHR from their morphological studies. However, the present study showed that the a/e ratios in the SHR were similarly smaller both in the outer and inner cortexes than those in the WKY rats (−28% and −29%, respectively, compared with the WKY rats). These observations clearly showed that the arterioles in the inner cortex function in response to the pressure, similar to the arterioles in the outer cortex. This assumption is consistent with a study using an isolated, juxtamedullary nephron preparation in which juxtamedullary nephrons sufficiently autoregulate glomerular pressure. A recent report using hydropnephrotic kidney showed that male rats' juxtamedullary arterioles have autoregulation but that female rats' juxtamedullary arterioles do not.

The present cast study revealed that when hypertension is established in SHR, glomerulosclerosis develops exclusively in the inner cortex, although the afferent arterioles constrict and the efferent arterioles dilate in the inner cortex almost to the same extent as in the outer cortex. There are some possible explanations for this. First, the glomeruli in the inner cortex might be exposed to higher pressure than those in the outer cortex; thus, the glomerular pressure rises in spite of the lower a/e ratio, and glomerulosclerosis develops. No evidence for this is available at present. Second, as a cause of glomerulosclerosis, factors other than glomerular hemodynamics might play an important role. Recently, some nonhemodynamic factors such as reactive oxygen species and glomerular hypertrophy have been shown to be keys for the development of glomerulosclerosis. Thus in the SHR, such nonhemodynamic factors might also play important roles in the development of glomerulosclerosis. Further study is necessary before we understand the development mechanism of glomerulosclerosis in view of the regional difference.

In summary, both the afferent and efferent arterioles change to protect glomeruli from hypertensive damage in response to a rise in the perfusion pressure, and sufficient systemic pressure reduction abolishes these arteriolar changes. Although the arteriolar changes occur similarly in the outer and inner cortexes, glomerulosclerosis is prominent in the inner cortex. This inconsistency between arteriolar changes and the development of glomerulosclerosis should be solved in future study, which will reveal the pathogenesis of glomerulosclerosis in SHR.

Acknowledgments

We thank Sanae Ogawa for her technical assistance, and Dr. Poul Faarup, University Institute of Pathological Anatomy, University of Copenhagen, Copenhagen, Denmark, for reviewing the manuscript.

References


KEY WORDS • kidney • glomerulosclerosis • arteriole • microvascular cast • spontaneously hypertensive rat

[Note: The image contains a URL and a date, which are not relevant to the text content.]
Renal arteriolar diameters in spontaneously hypertensive rats. Vascular cast study.
K Kimura, A Tojo, H Matsuoka and T Sugimoto

Hypertension. 1991;18:101-110
doi: 10.1161/01.HYP.18.1.101

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/18/1/101

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/