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 glomerulopathy 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), trichlormethia-
zide (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 experi-
ments, 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\(^{17}\) 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 consid-
ered to reach the effective pharmacological level.\(^{17}\)

The body weight and blood pressure were mea-
sured three times during the experiment: before drug
treatment (at 4 weeks old), after 2 weeks of admin-
istration 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
(Natsume 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.\(^{10-12}\) Under pentobarbital
anesthesia, a polyethylene catheter (PE-60) was in-
serted retrogradely into the abdominal aorta, and the
systemic blood pressure was monitored via the cath-
eter by using a three-way stopcock, which was con-
ected to an electric manometer (Nihon Koden Co.,
Tokyo). Immediately after the proximal aorta was
ligated between the right and left renal arteries and
the left renal vein was opened by a small incision for
outflow, 0.9% saline was infused at room tempera-
ture for a few seconds, followed by 2.5% glutaral-
dehyde in 0.1 M phosphate buffer at pH 7.4 for 3
minutes for fixation of the left kidney. The infusion
was performed by a hand syringe and the infusion
pressure was adjusted to be equal to the mean
arterial pressure measured just before the ligation of
the proximal aorta.\(^{10}\) After the fixation, the acryl
resin (Mercox, Dai-Nihon Inki Co., Tokyo) was in-
fused to make a cast of the vascular system in the left
kidney.

A portion of the renal tissue was examined by light
microscopy. The rest of the tissue was incubated at
room temperature for 24 hours so that the vascular
casts cured sufficiently. The renal tissue was then
digested and removed in 30% sodium hypochlorite
solution at room temperature overnight. Casts were
then transferred to fresh sodium hypochlorite, and
remnant renal tissue was completely removed in 2
hours. The casts were rinsed with distilled water
several times and dried.

The glomerular casts were subsequently dissected
under a stereomicroscope (Nikon Co., Tokyo). Cast-
ing 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 de-
fects in capillary tufts; therefore, sclerotic glomeruli
were excluded from the evaluation because once
glomeruli become sclerotic, the glomerular hemody-
namics and thus the arteriolar diameters are proba-
bly greatly altered. We assumed that only nonscle-
rotic glomeruli should be evaluated to clarify the
relation of arterioles to the development of hyper-
tensive 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 in-
er cortical glomeruli, interlobular arteries were ar-
bitrarily divided into upper and lower parts. Glo-
meruli 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 arteri-
oles originated directly from arcuate arteries were
included in the inner cortical glomeruli. The casts
were mounted on stubs for scanning electron micro-
scopic 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)
was used for examining and photographing the casts.

Casts were examined in the scanning electron
microscope at 600\( \times \) using an accelerating voltage of
20 kV and a working distance of 15 mm. The diameters
of arterioles were estimated on the photo-
graphic prints as previously described.\(^{16,18}\) In a seg-
ment of arteriole 50 \( \mu m \) from the glomerulus, the
diameter was measured at five points and a mean vessel diameter of the arteriole was determined by averaging all measurements. The mean arteriolar diameters for each rat were calculated separately in the outer and inner cortices and also in the whole cortex by averaging mean vessel diameters of the arterioles. These mean values for each rat were then pooled and the mean vessel diameters for each group were calculated and submitted to analysis.

Although the purpose of the present study was simultaneous evaluation of both afferent and efferent arterioles, only a small number of glomerular casts were accompanied by both afferent and efferent arteriolar casts as mentioned above; thus, we could not exclude the possibility that the arterioles used in the present study did not represent the general population of arterioles in the kidney. Therefore, in the WKY and SHR groups of 20-week-old rats we also measured the diameters of the afferent arterioles that had no corresponding efferent arterioles and observed whether there were significant differences in diameters between these afferent arterioles and the afferent arterioles with corresponding efferent arterioles. The total number of glomerular casts examined in each cortical region in each group ranged from 50 to 82.

**Glomerulosclerosis Score**

For the semiquantitative evaluation of hypertensive glomerular damage, the glomerulosclerosis score was defined as previously described. On the periodic-acid-Schiff–stained light microscopic specimens, approximately 50 glomeruli from the outer cortex and the same number of glomeruli from the inner cortex for each kidney were graded according to the degree of sclerosis: 0, if no mesangial expansion; 1, if mild mesangial expansion (less than 30% of a glomerular area); 2, if moderate mesangial expansion (30–60% of a glomerular area); 3, if marked mesangial expansion (more than 60% of a glomerular area); and 4, if the sclerosis was global. This was performed by one observer (K.K.) in a blind fashion using coded slides. A weighted composite sclerosis score was then calculated for each kidney according to the following formula: glomerulosclerosis score=[1×(number of grade 1 glomeruli) + 2×(number of grade 2 glomeruli) + 3×(number of grade 3 glomeruli) + 4×(number of grade 4 glomeruli)]×100/(number of glomeruli observed).

**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, 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<0.0088; 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 effenter 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 effenter 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 effenter 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).
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

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 trichloromethiazide (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 trichloromethiazide (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></td>
<td>6 weeks old</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>Effferent</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>af/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>Effferent</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>af/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>20 weeks old</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>15.4±0.4</td>
</tr>
<tr>
<td>Effferent</td>
<td>11.2±0.6</td>
<td>13.3±0.6‡#</td>
<td>12.7±0.8</td>
<td>13.4±0.8</td>
</tr>
<tr>
<td>af/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>&quot;Afferent&quot;</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>Effferent</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>af/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>&quot;Afferent&quot;</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, trichlorothiazide (1 mg/kg/day) and hydralazine (20 mg/kg/day)-treated rats; afferent, afferent arteriolar diameters (μm); effferent, effferent arteriolar diameters (μm); af/ef, ratio of afferent to effferent 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 effferent arteriole. Analysis of outer cortex vs. inner cortex was by Student's t test; all other analyses were by Bonferroni method.

*a<p<0.004 and †p<0.0008 vs. WKY, SHR, and SHR+TH.
#p<0.004 vs. SHR.
$p<0.004 vs. SHR+CAP and SHR+TH.
††p<0.004 vs. WKY.
§§p<0.004 vs. outer cortex vs. inner cortex.
**p<0.004 vs. WKY, SHR+CAP, and SHR+TH.
†††p<0.0008 vs. WKY, SHR+CAP, 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 diameters were approximately equal in the WKY and SHR groups; the a/e ratio was largest in the SHR+CAP group. At 20 weeks old, the afferent arteriolar diameters in the SHR group were smaller than that of other groups in the outer and inner cortices. The effferent arteriolar diameters in the SHR in the outer cortex were larger than that of the WKY group. The a/e 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 effferent arteriolar diameters in the inner cortex were significantly larger than that in the outer cortex. The a/e 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 effferent arterioles. The afferent arterioles that had no corresponding effferent arterioles were studied at 20 weeks old (shown as "afferent") (Table 1). They showed diameters similar to those of the afferent arterioles with corresponding effferent 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 largest 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 2. Glomerulosclerosis Scores 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>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>
<tr>
<td><strong>20 weeks old</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outer cortex</td>
<td>28 ± 4</td>
<td>36 ± 2*</td>
<td>37 ± 6</td>
<td>25 ± 3</td>
</tr>
<tr>
<td>Inner cortex</td>
<td>29 ± 5</td>
<td>63 ± 10†</td>
<td>37 ± 7‡</td>
<td>26 ± 2</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.

*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.

**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 pregglomerular arteriole plays an important role in maintaining the glomerular pressure (autoregulation). When the perfusion pressure falls, pregglomerular 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 trichlormethiazide 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). Dwarkin 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 cortices, and regional differences were studied. There was no qualitative difference between the arteriolar changes in the outer and inner cortices 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 higher 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 cortices 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 hydrourethrocystic 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 cortices, 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
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/