Vascular Hypertrophy in Renal Hypertensive Spontaneously Hypertensive Rats

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Abstract Vascular smooth muscle cells isolated from spontaneously hypertensive rats (SHR) replicate faster in vitro than do cells from Wistar-Kyoto (WKY) rats, suggesting that the vascular hypertrophy seen early in the life of SHR might be at least partially caused by abnormal cellular growth properties in vivo. To test whether specific growth stimuli produce more extensive hypertrophy in SHR than WKY rats, we compared their cardiovascular growth responses to two-kidney, one clip renal hypertension. Six-week-old animals were subjected to either renal artery clipping or sham operation. Four weeks after renal artery clipping, there was a proportionately smaller rise in systolic blood pressure in SHR than WKY rats (21% and 44%, respectively); however, the overall level of systolic blood pressure achieved in the two rat strains differed by less than 10 mm Hg (4%). Limitations in the blood pressure responses of SHR to renal artery clipping were not due to inadequate development of left ventricular hypertrophy, as this was greater in SHR than WKY rats; however, aortic hypertrophy was similar in both strains. Aortic DNA content changes in SHR were consistent with a significant hyperplasia of medial smooth muscle cells, whereas in WKY rats, there was cellular hypertrophy. Small and medium-sized arteries of the mesenteric vasculature were also hypertrophied in SHR, and the medial cross-sectional area increased by 63% and 114%, respectively, compared with increases of only 15% and 23% in WKY rats. Strain differences between the sham-operated rat groups were small. In a hemodynamic analysis of the hindquarter vasculature performed under constant flow conditions, an increase in vascular resistance was consistent with encroachment of the vessel wall on the lumen. Average hindquarter vascular lumen diameter appeared smaller in SHR than WKY rats, and the increase in perfusion pressures during maximal constriction with methoxamine plus angiotensin II in both rat strains also suggested significant vessel wall hypertrophy. The increase in perfusion pressure at maximal constriction was greater in SHR than WKY rats, and the absolute level of systolic blood pressure could not account for this difference. Overall, this study indicates that the cardiovascular system of the SHR is more responsive to the hypertrophic stimuli of two-kidney, one clip renal hypertension. The more rapid and in some vessels more extensive hypertrophy that develops in SHR compared with WKY rats, despite only small differences in systolic blood pressure, supports the hypothesis that the stimulation of genes that contribute to the abnormal growth of vascular smooth muscle cells of the SHR may contribute to the development of vascular hypertrophy in these animals. (Hypertension. 1994;24:8-15.)

Key Words • hypertension, Goldblatt • rats, inbred SHR • heart hypertrophy • vascular resistance • muscle, smooth, vascular • polyploidy

The presence of cardiovascular hypertrophy is well demonstrated in both human and experimental hypertension and plays an important role in the maintenance of elevated blood pressure. In renal hypertension the "cardiovascular amplifiers" undoubtedly play an important homeostatic role in elevating arterial blood pressure to maintain adequate renal function. Cardiovascular hypertrophy and the associated amplifiers may not only develop in response to a sustained elevation in blood pressure but also in some circumstances appear to develop independently of any abnormal rise in blood pressure. For example, in spontaneously hypertensive rats (SHR) cardiovascular hypertrophy is present very early in development, and it has been suggested that this may play a role in initiating the hypertension. However, although the early abnormal changes in cardiovascular structure in SHR have been carefully documented, the mechanisms responsible for these changes are yet to be defined.

A number of in vitro studies have demonstrated that smooth muscle cells cultured from SHR aorta replicate more rapidly and are less responsive to some growth inhibitors than cells cultured from Wistar-Kyoto (WKY) rat aorta. Thus, it is possible that the abnormal expression of genes controlling cellular growth in vascular smooth muscle cells from SHR contributes to the vascular hypertrophy in vivo. During development and growth of SHR, the increasing blood pressure and greater demands for blood flow might stimulate greater smooth muscle growth and thus promote development of a larger "vascular amplifier" in these animals. One question of interest is whether the cardiovascular hypertrophy and/or vascular amplifiers in SHR are close to maximal development or whether the superimposition of additional hypertension, for example, renovascular hypertension, could cause an exaggerated growth response relative to the renal hypertensive WKY rat. Two-kidney, one clip (2K1C) renal hypertension is largely initiated through increased activity of the renin-angiotensin system; angiotensin II (Ang II) is known to promote the growth of smooth muscle cells and participate in the development of cardiac hypertrophy. Hence, in the present study we compared the effects of 2K1C renal hypertension on the blood pressure and variety of indexes of cardiovascular structure in SHR and WKY rats.
Methods

Animal Operations and Blood Pressure Measurements

We used male SHR and WKY rats from colonies established at the Baker Institute from Japanese stock. 18 2K1C renal hypertension was induced in 6-week-old SHR and WKY rats by application of a 0.20-mm inner diameter silver clip to the left renal artery with rats under sodium methohexital anesthesia (20 mg/kg body wt IP). Age-matched control rats were sham operated by exposing the renal artery. Surgical procedures and animal care conformed to the National Health and Medical Research Council of Australia guidelines and were approved by our Institutional Animal Ethics Committee. Two groups (2K1C and sham-operated) were examined in each strain 4 and 8 weeks after surgery. Systolic blood pressure (SBP) was measured in conscious animals by tail-cuff plethysmography (ITTC Life Science Instruments) while the animals were in a restriction chamber at 27°C. 4 Beginning 2 weeks before surgery, SBP was measured twice weekly until the end of the experiments. The two values from each week were averaged for each animal.

Tissue Collection

Before tissue was collected, the rats were deeply anesthetized with pentobarbital (60 mg/kg IP), and the abdominal aorta was cannulated proximal to the bifurcation for perfusion of the vasculature. Immediately before the various perfusion studies (see below) were begun, the heart and descending thoracic aorta (between the left subclavian artery and diaphragm) were removed and weighed. The aorta was cleaned of adhering fat and loose connective tissue. In the 8-week rat groups the aorta was also stripped of intercostal arteries and closely adhering connective tissue.

Morphometry

Vessels were taken from 10 animals in each group after 4 weeks of renal hypertension and from 6 animals after 8 weeks of renal hypertension for morphometric analysis. In brief, the abdominal aorta was cannulated as described above and the mesenteric circulation perfused with Hanks' balanced salt solution containing papaverine HCl (0.1 mg/mL) and heparin (5 U/mL) for 4 to 5 minutes at a flow rate of 1 mL/min per 100 g body weight. Glutaraldehyde (2.5%) in 0.1 mol/L phosphate buffer (pH 7.4) was then perfused for 10 minutes at the same flow rate. The abdominal aorta and mesenteric bed were removed into fixative overnight, then samples of aorta were closely adhering connective tissue. These tissues were washed, then postfixed in 1% osmium tetroxide for 2 hours, dehydrated in alcohol, and embedded in epoxy resin (Polarbed, Bio-Rad) for sectioning. Cross sections from the abdominal aorta and mesenteric vessels were cut 0.5 μm thick and stained with toluidine blue in 1% borax. The mesenteric arteries sampled (see Fig 1) were those branches that directly entered the intestine from the mesentery (type 1; approximately 150-μm lumen diameter) and the third-order branches proximal to these (type 5; approximately 250-μm lumen diameter). Three type 1 vessels and two type 3 vessels were taken from animals at 4 weeks after surgery; at 8 weeks only three type 3 vessels were sampled. Images of these sections were projected onto a digitizing tablet (Complot, Bausch and Lomb), and areas bounded by inner and outer elastic laminae were digitized and calculated by a morphometry software package (Capricorn Scientific Software). Medial area was determined as the difference between the two values.

Smooth Muscle Cell Isolation and Flow Cytometry

The thoracic aortas from eight animals in each group were stripped of periadventitial fat and connective tissues, blotted dry, and weighed. A 1-cm section proximal to the diaphragm was removed, frozen in liquid nitrogen, and stored at —70°C for DNA analysis (see below). The remaining piece of thoracic aorta was deendothelialized by wiping the intimal surface with a cotton swab. The aortas were then incubated with collagenase (200 U/mL, Worthington Biochemical Co) in Dubecco's modified Eagle medium for 30 minutes at 37°C. After mechanical removal of the adventitia, the media was minced and incubated with fresh collagenase at 37°C for a further 2 hours. After this time elastase (10 U/mL, Sigma Chemical Co) was added, and with periodic trituration a uniform single cell suspension was obtained (approximately 1 hour). The dispersed cells were centrifuged at 800 rpm for 8 minutes and resuspended in phosphate-buffered saline, and the suspension was filtered through 50-μm plastic mesh to remove any undigested fragments. Cell diameter was measured on a Coulter counter with cell size attachment and expressed as the median value derived from the cell size distribution. The size of polyploid cells could not be determined from this protocol. For flow cytometry measurement of cellular DNA content, ethidium bromide was added to the cell suspension (50 μg/mL), and cells were stored at —70°C in the presence of 10% dimethyl sulfoxide. The proportion of polyploid cells in the suspension was estimated on samples of at least 10,000 cells with a Fluorescence Activated Cell Sorter (FACS II, Becton Dickin

Fig 1. Schematic representation shows segments of mesenteric vasculature used for morphometric assessment of hypertrophy of small muscular arteries in renal hypertensive and sham-operated spontaneously hypertensive and Wistar-Kyoto rats. SMA indicates superior mesenteric artery.

DNA Content of Thoracic Aorta

DNA content in samples of thoracic aorta from the different rat groups was measured according to the method of Labarca and Paigen. 19 Briefly, five samples from each group were thawed in 0.1 mol/L phosphate-buffered saline and their lengths measured. The adventitia was stripped from the vessels and the remaining media weighed before being homogenized in a glass tissue grinder with 0.05 mol/L phosphate buffer and 2 mol/L NaCl at pH 7.4. The resulting homogenate was sonicated briefly and then centrifuged at 10,000g for 20 minutes. The fluorescent dye bis-benzimide (Hoechst 33258) was added to the supernatant, and fluorescence (excitation, 356 nm; emission, 458 nm) was read in a fluorometer (Perkin-Elmer) against purified calf thymus DNA standards (Sigma).

Hindquarter Resistance Properties

We measured perfusion pressures of the hindquarter vasculature during maximal dilatation (PPhmax) and maximal constriction (PPmin) in 7 to 12 animals from each group, essentially as described previously. 20 Briefly, animals were anesthetized with sodium pentobarbital and placed in a warm chamber where rectal temperature was maintained at 36° to 38°C. The aortic bifurcation was exposed through a midline incision and...
the abdominal aorta cannulated with a 1.5-mm cannula. The animals were heparinized (1000 IU/kg) and the middle caudal and caudal mesenteric arteries ligated. The hindquarters were perfused at 10 mL/min per 100 g hindquarter weight with modified Tyrode's solution containing 1.5% dextran 70.20 Flow of perfusate was begun immediately after transection of the spinal cord and was maintained for approximately 5 minutes to flush blood from the hindquarters before papaverine HCl was given as a bolus (1.5 mg/100 g body wt) and PP_max was measured. After a 20-minute washout period we obtained perfusion pressure–response curves to increasing concentrations of methoxamine (0 to 50 µg/mL) and finally to maximal constriction (PP_max) with a bolus dose (50 µg) of Ang II.*

**Statistics**

Group comparisons were made according to strain and treatment by one-way ANOVA.21 The between-groups sums of squares were partitioned orthogonally into single degrees of freedom to compare the different rat groups over the two time periods, ie, 4 and 8 weeks after the operations. Data are presented as mean±SEM. A value of P<.05 was considered statistically significant.

**Results**

**Blood Pressures and Body Weights**

Preoperative SBP values were similar in the young SHR and WKY rats, averaging 121 and 121 mm Hg 1 week before the operations (P>.05 for difference) and 134 and 120 mm Hg, respectively, during the week of the operations (P<.05 for differences, Fig 2). In sham-operated SHR (SHR SH) SBP continued to rise with age, and at 10 weeks of age SBP averaged 190 mm Hg; this pressure was maintained for the duration of the study. By contrast, in sham-operated WKY rats (WKY SH), there was only a small rise in SBP, which stabilized at approximately 140 mm Hg by 8 weeks of age (Fig 2).

After renal artery clipping SBP rose more rapidly to levels above those observed in sham-operated rats in each strain. In 2K1C SHR (SHR 2K1C), SBP 4 weeks after clipping was approximately 40 mm Hg higher than in SHR SH, and this difference was approximately the same when the animals were 14 weeks of age, ie, approximately 8 weeks after clipping. Corresponding elevation of SBP in 2K1C WKY rats (WKY 2K1C) was 70 mm Hg at 4 weeks and approximately 77 mm Hg 8 weeks after clipping. Absolute levels of SBP at 8 weeks after clipping averaged 225 mm Hg in SHR 2K1C and 219 mm Hg in WKY 2K1C; corresponding values in SHR SH and WKY SH were 190 and 140 mm Hg, respectively. Thus, the induction of 2K1C renal hypertension abolished most of the difference in SBP between SHR SH and WKY SH.

Body weights in WKY rats were relatively unaffected by renal hypertension (Fig 2). In SHR 2K1C, body weights were also well maintained. However, 6 weeks after the onset of renal hypertension, body weights of SHR 2K1C tended to be maintained rather than rise at the same rate as those of SHR SH.

**Heart Weights and DNA Content**

Four weeks after sham operation both the left ventricular (LV) weights and the ratios of the left ventricle to body weight (LV/BW) were higher in SHR than WKY rats (P<.05 for differences) (Table 1). LV weights increased with age after sham operation, and by the 8th week the increase averaged 12% in both strains. At this time LV/BW was 30% greater in SHR SH than WKY SH.

The effect of renal clipping on the heart was greater in SHR. Four weeks after clipping, LV/BW in SHR 2K1C was 54.5% greater than in SHR SH, and at 8 weeks it was 82.3% greater (Table 1). In WKY 2K1C 4 weeks after clipping the ratio was 41% greater than in WKY SH, and at 8 weeks it was 57% greater. DNA content was measured only at 4 weeks after clipping; it was similar in SHR SH and WKY SH, and the increase caused by clipping was approximately the same in both strains (40.6% in SHR, 36.5% in WKY rats). At 4 and 8 weeks LV/BW was 24.5% and 50.8% higher in SHR 2K1C than in WKY 2K1C (P<.05 for differences). The ratio of DNA to body weight was also similar in the two strains and was unaffected by renal artery clipping.

A small but statistically significant increase in right ventricular weight could be detected only in SHR 2K1C during the 8th week of renal hypertension (Table 1).

**Aortic Structure**

After sham operation the abdominal aortic medial cross-sectional areas and the ratios of thoracic aortic to body weight (A/BW) were similar in the two strains (Table 2). In each strain 2K1C renal hypertension caused a marked increase in A/BW (P<.01) and medial cross-sectional area that averaged approximately 30% (P<.01). There were no differences in these responses of the two strains to renal hypertension at either 4 or 8 weeks (Table 2).

**Mesenteric Vessels**

The small (10%) difference in medial cross-sectional areas of the type 1 mesenteric vessels of SHR SH and WKY SH 4 weeks after operation was not statistically significant (P>.05, Table 2). At 4 weeks the cross-sectional area in SHR 2K1C was increased by 61% (P<.01), whereas
TABLE 1. Cardiac Weights, DNA, and Body Weights of SHR and WKY Rats 4 and 8 Weeks After Renal Artery Clipping or Sham Operation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time Interval, wk</th>
<th>SHR</th>
<th>2K1C</th>
<th>WKY</th>
<th>2K1C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricle weight, mg</td>
<td>4-5</td>
<td>762±9</td>
<td>(13)</td>
<td>1138±15*</td>
<td>(9)</td>
</tr>
<tr>
<td></td>
<td>7-8</td>
<td>849±18</td>
<td>(8)</td>
<td>1250±62*</td>
<td>(7)</td>
</tr>
<tr>
<td>Left ventricle/body weight, mg/g</td>
<td>4-5</td>
<td>2.99±0.03</td>
<td>(13)</td>
<td>4.62±0.09*</td>
<td>(9)</td>
</tr>
<tr>
<td></td>
<td>7-8</td>
<td>2.88±0.05</td>
<td>(8)</td>
<td>5.25±0.39*</td>
<td>(7)</td>
</tr>
<tr>
<td>Left ventricle DNA, μg</td>
<td>4-5</td>
<td>1183±70</td>
<td>(10)</td>
<td>1663±81*</td>
<td>(10)</td>
</tr>
<tr>
<td></td>
<td>7-8</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Left ventricle DNA/left ventricle weight, μg/mg</td>
<td>4-5</td>
<td>1.62±0.08</td>
<td>(10)</td>
<td>1.48±0.07</td>
<td>(10)</td>
</tr>
<tr>
<td></td>
<td>7-8</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Right ventricle weight, mg</td>
<td>4-5</td>
<td>172±7</td>
<td>(13)</td>
<td>183±7</td>
<td>(15)</td>
</tr>
<tr>
<td></td>
<td>7-8</td>
<td>185±5</td>
<td>(8)</td>
<td>237±18*</td>
<td>(7)</td>
</tr>
<tr>
<td>Right ventricle/body weight, mg/g</td>
<td>4-5</td>
<td>0.68±0.03</td>
<td>(13)</td>
<td>0.74±0.03</td>
<td>(9)</td>
</tr>
<tr>
<td></td>
<td>7-8</td>
<td>0.63±0.02</td>
<td>(8)</td>
<td>1.01±0.11*</td>
<td>(7)</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>4-5</td>
<td>255±2</td>
<td>(13)</td>
<td>245±3</td>
<td>(15)</td>
</tr>
<tr>
<td></td>
<td>7-8</td>
<td>307±6</td>
<td>(8)</td>
<td>262±11*</td>
<td>(7)</td>
</tr>
</tbody>
</table>

SHR indicates spontaneously hypertensive rats; WKY, Wistar-Kyoto; 2K1C, two-kidney, one clip hypertensive; and nd, not done. Values in parentheses indicate n.
*P<.05 vs corresponding sham.
†P<.05 vs 2K1C WKY rats.

An essentially similar profile of responses to 2K1C renal hypertension was observed in the type 3 mesenteric vessels at both 4 and 8 weeks. In SHR, the medial in WKY the increase was only 22%. Mean cross-sectional areas in SHR and WKY averaged 11.8 and 8.1×10^3 μm^2, respectively (P<.01, Table 2).

TABLE 2. Abdominal Aorta and Mesenteric Vessel Media Cross-sectional Area at 4 and 8 Weeks of Renal Hypertension in SHR and WKY Rats

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Time, wk</th>
<th>SHR</th>
<th>2K1C</th>
<th>WKY</th>
<th>2K1C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal aorta, 10^6 μm^2</td>
<td>4-5</td>
<td>308±17</td>
<td>400±42*</td>
<td>313±19</td>
<td>407±39*</td>
</tr>
<tr>
<td></td>
<td>7-8</td>
<td>301±10</td>
<td>364±24*</td>
<td>277±10</td>
<td>451±27*</td>
</tr>
<tr>
<td>Mesenteric arteries</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 3, 10^6 μm^2</td>
<td>4-5</td>
<td>19.3±1.6</td>
<td>41.3±2.7*</td>
<td>16.2±0.9</td>
<td>16.8±1.7</td>
</tr>
<tr>
<td></td>
<td>7-8</td>
<td>15.9±1.5</td>
<td>29.8±4.4*</td>
<td>11.5±1.0</td>
<td>21.4±1.6*</td>
</tr>
<tr>
<td>Type 1, 10^6 μm^2</td>
<td>4-5</td>
<td>7.32±0.66</td>
<td>11.81±0.90*</td>
<td>6.65±0.75</td>
<td>8.14±0.82*</td>
</tr>
<tr>
<td></td>
<td>7-8</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

Definitions are as in Table 1. n=10 animals for 4-5-week groups and 6 animals for 7-8-week groups.
*P<.05 vs corresponding sham.
cross-sectional areas were 19% and 38% greater than in WKYSH 4 and 8 weeks after the operations, respectively (P<.05, Table 2, Fig 3). In SHR2KIC medial cross-sectional areas were 114% and 87% greater than SHR SH after 4 and 8 weeks of renal hypertension (P<.01); in WKYSKIC increases in the cross-sectional area of the media after 4 and 8 weeks averaged 4% and 86%, respectively (P<.01). After 8 weeks of renal hypertension mean cross-sectional areas in SHR2KIC were nearly 40% greater than in WKYSKIC (29.8 and 21.4×10^-3 μm^2, respectively; P<.01, Table 2).

### Aortic Smooth Muscle Cell Proliferation and Hypertrophy

To examine whether the increases in aortic hypertrophy were caused by vascular smooth muscle cell proliferation, hypertrophy, or polyplody, we measured DNA content of the media of the vessel as well as cell size and nuclear polyplody of enzyme-dispersed smooth muscle from the thoracic aorta of rats 4 weeks after the induction of renal hypertension or sham operation.

DNA content and nuclear polyplody were greater in SHRSH than WKYSH (Table 3). After 4 weeks of renal hypertension, DNA content of the thoracic aorta media increased by 30% in SHR2KIC compared with SHRSH (P<.01, Table 3). In contrast, in WKYSKIC the increase in DNA content was small (10%) and not statistically significant (Table 3). The increase in cell polyplody in SHR2KIC could account for only 7.5% of the increase in DNA, suggesting significant smooth muscle cell hyperplasia in the aorta of these animals. In the WKYSKIC the increase in polyplody averaged 12% (Table 3), accounting for all of the increase in DNA content. Cellular hypertrophy was greater in the WKYSKIC than SHR2KIC. The average aortic smooth muscle cell diameter from WKYSKIC was increased by 12% (P<.05) relative to the sham animals; in SHR2KIC the increase was only 3% (P<.05).

### Hindlimb Vascular Resistance Properties

Because the rate and extent of cardiovascular remodeling in SHR and WKY rats differed during 2K1C hypertension, we also investigated the time course of changes in hindlimb vascular reactivity. Increases in PP_{max} and PP_{min} of resistance vessels are known to be closely associated with increases in the wall-to-lumen ratio.1

In SHRSH, PP_{min} was on average 4% (P>.05) and 16% (P<.05) greater than in WKYSH 4 and 8 weeks after the operations (Table 4). After 4 weeks of renal hypertension in SHR2KIC, PP_{min} was increased markedly by 20% from 26.0 to 31.3 mm Hg (P<.01). A 13% increase was observed 4 weeks after renal artery clipping in WKYSKIC. However, in these animals PP_{min} was on average 3.2 mm Hg lower than in the SHR2KIC (31.3 mm Hg versus 28.1 mm Hg in WKYSKIC, P<.01). After 8 weeks of renal hypertension, PP_{min} increased only slightly in SHR2KIC to 34.3 mm Hg (Table 4); in WKYSKIC the increase was substantially greater so that the difference between the two groups of animals (1.6 mm Hg) was no longer statistically significant (34.3 versus 32.7 mm Hg, SHR2KIC versus WKYSKIC).

PP_{max} measured after 4 weeks of renal hypertension was 19% greater in SHRSH (338±7 mm Hg) than WKYSH (283±4 mm Hg) but only 11% higher in both SHR2KIC and WKYSKIC when compared with their respective sham-operated controls (Table 4). During the 8th week of renal hypertension, PP_{max} in SHRSH averaged 339 mm Hg compared with 297 mm Hg in WKYSH (P<.01). In SHR2KIC, PP_{max} was elevated by 28% com-

### Table 3. Aortic DNA Content and Aortic Smooth Muscle Cell Size and DNA Content In SHR and WKY Rats After 4 Weeks of Renal Hypertension

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SHR</th>
<th>WKY Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>2K1C</td>
</tr>
<tr>
<td>Thoracic aorta DNA content, μg/cm</td>
<td>11.8±0.5 (5)</td>
<td>15.4±0.4* (5)</td>
</tr>
<tr>
<td>Smooth muscle cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyplodity, % 4N cells</td>
<td>5.4±0.1 (7)</td>
<td>12.9±0.3* (11)</td>
</tr>
<tr>
<td>Cell diameter, μm</td>
<td>13.1±0.1 (7)</td>
<td>13.5±0.1* (9)</td>
</tr>
</tbody>
</table>

SHR indicates spontaneously hypertensive rats; WKY, Wistar-Kyoto; and 2K1C, two-kidney, one clip hypertension. Values in parentheses indicate number of animals.

*P<.05 vs corresponding sham.
pared with 24% in WKY 2K1C. However, despite a not very different percent increase in \( \Delta P \), between the two renal hypertensive rat strains, \( \Delta P \) values in SHR 2K1C at 4 and 8 weeks were on average 64 and 65 mm Hg higher, respectively, than in WKY 2K1C (\( P < .01 \) for difference).

**Discussion**

In the vasculature, wall thickness increases in proportion to the severity of the hypertension so that circumferential wall stress is maintained relatively constant.\(^{22,23}\) We used this relation to assess whether the responsiveness of the vasculature of SHR to the hypertrophic stimulus of 2K1C renal hypertension is greater than that of WKY rats. Our findings indicate that despite the elevation of blood pressure induced by the renal hypertension in WKY rats being greater than that in SHR, the early vascular hypertrophy associated with 2K1C renal hypertension developed more rapidly in SHR. Initial increases in the medial mass of the aorta were similar in the two strains; however, in SHR 2K1C it was predominantly the consequence of smooth muscle cell proliferation, whereas in WKY 2K1C cellular hypertrophy accounted for much of this increase in media mass. Vascular hypertrophy was most prominent in the mesenteric vessels of SHR 2K1C and in absolute terms remained greater than that in WKY rats with chronic 2K1C hypertension. In the hindquarter vasculature there was evidence for passive average lumen narrowing during the early stages of renal hypertension, and hypertrophy also appeared to develop, as suggested by the increase in \( \Delta P \). Overall, our observations suggest that vascular smooth muscle of SHR is more responsive to the hypertrophic stimuli of 2K1C renal hypertension. However, the heterogeneous pattern of development of vascular hypertrophy in the aorta, hindquarters, and mesenteric vessels is consistent with a number of different mechanisms being activated in response to “reindependent” hypertension.

Qualitatively, renal artery clipping in the two rat strains produced essentially similar effects on SBP. In WKY 2K1C, SBP increased rapidly after renal artery clipping, and by the end of the experimental interval it was only 10 mm Hg (4%) lower than in age-matched SHR 2K1C. The average rise in SBP induced by renal artery clipping of SHR 2K1C was only 50% of the pressure rise observed in WKY 2K1C. Because 2K1C renal hypertension is renin dependent in the early stages,\(^6\) it is possible that differences in plasma renin levels in the two rat strains in response to renal artery constriction account for the smaller blood pressure elevation in SHR 2K1C; alternatively, other homeostatic mechanisms could also be influencing the level to which SBP rises in SHR 2K1C. The level to which cardiac hypertrophy develops does not appear to influence the final SBP response because in SHR 2K1C LV hypertrophy was far greater than in WKY 2K1C. Right ventricular hypertrophy was only evident in SHR 2K1C during the later stages of renal hypertension, possibly indicative of some mild impairment of cardiac function.

Many years ago Wolinsky\(^{24}\) demonstrated that the aortic hypertrophy that develops during long-term 2K1C renal hypertension is caused by an initial increase in smooth muscle mass followed by a profound increase in extracellular matrix. The increase in muscle mass tended to occur during the first 2 weeks of the renal hypertension, and after this time the major component of the increase in wall thickness was due to increases in the production of extracellular matrix.\(^{22,24}\) We also observed a rapid development of aortic hypertrophy in SHR and WKY rats after the induction of 2K1C hypertension. Initially, the increase in cross-sectional area of the abdominal aorta was similar in the two strains of renal hypertensive rats but later was expressive of greater aortic hypertrophy in the WKY rats. Despite the initially similar aortic hypertrophy in the two renal hypertensive rat strains, the manner by which this was achieved differed markedly. In SHR 2K1C, large increases in DNA content, consistent with smooth muscle cell proliferation, accounted for much of the early increase in muscle mass. In WKY animals, 4 weeks of renal hypertension did not increase cell number but caused a substantial increase in smooth muscle cell size and a small increase in DNA content. Thus, it would appear that aortic smooth muscles of SHR in vivo also tended to proliferate more rapidly than those of WKY rats, an effect consistent with previous observations in cell culture.\(^6,9\) However, the lesser tendency of WKY vascular smooth muscle cells to proliferate does not appear to greatly limit the magnitude of aortic hypertrophy in these animals. Despite this, the fact that the increase in aortic wall thickness per unit increase in SBP was greater in SHR 2K1C than WKY 2K1C indicates that in SHR this vessel is more responsive to the hypertrophic stimulus of 2K1C renal hypertension. Other aspects of smooth muscle cell hypertrophy, such as the increase in the frequency of polyploid smooth muscle cells, although increased in the 2K1C renal hypertensive animals, were similar in the two rat strains. We did not examine whether the later changes in cross-sectional

### Table 4. Hindquarter Vascular Perfusion Pressures at Maximal Dilatation and Maximal Constriction of SHR and WKY Rats 4 and 8 weeks After Renal Artery Clipping or Sham Operation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time Interval, wk</th>
<th>SHR</th>
<th>2K1C</th>
<th>WKY Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P_{\text{max}} ), mm Hg</td>
<td>4-5</td>
<td>26.0±0.8 (10)</td>
<td>31.3±1.0*(11)</td>
<td>24.9±0.9 (12)</td>
</tr>
<tr>
<td></td>
<td>7-8</td>
<td>31.1±0.6 (9)</td>
<td>34.3±2.1*(9)</td>
<td>26.9±1.1 (7)</td>
</tr>
<tr>
<td>( P_{\text{min}} ), mm Hg</td>
<td>4-5</td>
<td>338±7 (4)</td>
<td>377±15*(4)</td>
<td>283±4 (3)</td>
</tr>
<tr>
<td></td>
<td>7-8</td>
<td>339±9 (9)</td>
<td>435±6*(9)</td>
<td>297±7 (7)</td>
</tr>
</tbody>
</table>

SHR indicates spontaneously hypertensive rats; WKY, Wistar-Kyoto; 2K1C, two-kidney, one clip hypertension; \( P_{\text{max}} \), perfusion pressure at maximal dilatation; and \( P_{\text{min}} \), perfusion pressure at maximal constriction. Values in parentheses indicate n.

\* \( P < .05 \) vs corresponding sham.

† \( P < .05 \) vs corresponding 2K1C.
areas of the abdominal aortas during the 8th week of 2K1C renal hypertension were due to increases in extracellular matrix or further increases in muscle mass. 

Hypertrophy of the mesenteric vasculature in SHR<KIC and WKY<KIC followed a different pattern of development compared with the aorta. Mesenteric vascular hypertrophy developed more rapidly in SHR<KIC. Small differences in the rate at which SBP increased in the two groups of renal hypertensive animals could not account for the more extensive hypertrophy in the mesenteric vasculature of SHR<KIC. The greater hypertrophic response in SHR<KIC also supports the hypothesis of a more extensive growth response to 2K1C renal hypertension in SHR compared with WKY rats. We have recently shown that SHR and WKY rats have similar increases in polyplody of mesenteric artery smooth muscle cells after 4 weeks of 2K1C renal hypertension,25 but we did not investigate whether an increase in muscle cell mass accounted for most of the vascular hypertrophy in the resistance vasculature. Further studies defining the changes in smooth muscle cell numbers, hypertrophy, and extracellular matrix proteins will be required to define precisely how vascular smooth muscle cells within the mesenteric resistance vessels of SHR and WKY rats differ in their responsiveness to renal hypertension.

In a hypertrophied vasculature the increase in the wall-to-lumen ratio greatly enhances any changes in vascular resistances when compared with a normal vasculature.26 The cardiac hypertrophy that develops in the 2K1C renal hypertensive animals can also contribute to the exaggerated changes in vascular resistance.27,28 In a constant-flow hindquarter perfusion such as that used in the present study, such increases in vascular resistances are reflected by an elevated perfusion pressure in the hypertensive rats. By the 8th week of renal hypertension in the SHR and WKY rats, both PPmn and PPmax were significantly increased compared with sham-operated animals. These findings are consistent with those in the other vessels, which indicated that vascular hypertrophy was well developed at this time. As in the mesenteric vasculature, the higher PPmax and PPmn values in SHR<KIC compared with WKY<KIC are consistent with a greater vascular hypertrophy response in SHR. Our finding that PPmn is elevated in the perfused hindquarter vasculature after 4 weeks of renal hypertension, together with readily detectable hypertrophy of the aorta and mesenteric vasculature, suggests early and extensive remodeling of the vasculature in these animals. Both hypertrophy of the vessel wall and encroachment of the vessel wall on the lumen appear to be involved in the restructuring of the vasculature. According to Poiseuille's law the small increase in PPmn we observed 4 weeks after the induction of 2K1C renal hypertension could be accounted for by only a 2% reduction in the average lumen radius of the hindquarter vasculature.

At present we can only speculate on the trophic stimuli responsible for the extensive vascular hypertrophy in SHR<KIC and WKY<KIC. Development of renal hypertension in these animals is associated with high plasma and vascular Ang II levels29, in cell culture studies Ang II is known to induce vascular smooth muscle cell hypertrophy and polyplody.30,31 Some of these growth responses are probably the consequences of Ang II inducing the smooth muscle cells to produce platelet-derived growth factor-A chain, basic fibroblast growth factor, and transforming growth factor-B1. mRNA levels for these growth factors are elevated in vascular smooth muscle exposed to Ang II.12,13 Transforming growth factor-β directly induces hypertrophy of smooth muscle cells.14-32 It is also a potent stimulator of extracellular matrix biosynthesis.32 Ang II has also been shown to potentiate the mitogenic effect of platelet-derived growth factor-BB on vascular smooth muscle cells17,33 and on occasion has been reported to induce the proliferation of aortic myocytes cultured from SHR.3,17 However, because Ang II infusion in vivo does not appear to stimulate proliferation of aortic smooth muscle cells from SHR,34 these observations in cell culture may apply to the properties of a small subpopulation of cells within the blood vessel wall. Thus, although Ang II can be implicated in the development of vascular hypertrophy in 2K1C renal hypertensive SHR and WKY rats, the diversity of the cellular responses observed among vessels from different vascular beds strongly suggests that additional factors, presumably those that may already be activated in the SHR during this phase of hypertension development, such as the trophic influence of the sympathetic nervous system,35 that interact with the renal hypertension growth stimulus to influence the extent of the response. The precise roles of such mechanisms in the growth responses of different vascular beds in these renal hypertensive animals remain to be elucidated.

In summary, the present study has demonstrated that vascular smooth muscle of SHR is more reactive to the growth stimuli induced by 2K1C renal hypertension. In the aorta the data were consistent with a predominant increase in cell number and a smaller increase in cell size and polyplody in the SHR, whereas in the WKY rat the data suggest increases only in cell size and polyplody. Our finding that the rates at which vascular hypertrophy develops in the different vascular beds differ, together with the heterogeneity in vascular growth responses, argues strongly against circulating Ang II being solely responsible for the vascular hypertrophy. Rather, additional local factors either acting alone or possibly interacting with Ang II would account for the diversity in vascular growth responses in renal hypertensive SHR and WKY rats. In the future it will also be of interest to determine the factors responsible for the more extensive LV hypertrophy in renal hypertensive SHR.

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