Structural and Reactivity Alterations of the Renal Vasculature of Spontaneously Hypertensive Rats Prior to and During Established Hypertension

John S. Smeda, Robert M.K.W. Lee, and James B. Forrest

The renal vasculatures of Wistar Kyoto spontaneously hypertensive rats (SHR), prior to (4–5 week) and during established hypertension (21 week) and those of age-matched Wistar Kyoto normotensive rats (WKY) were morphometrically and pharmacologically studied. Under dilated conditions, the vascular resistances (RVR) of the isolated kidneys of young and adult SHR were similar to WKY. Morphometric measurements of renal vasculature indicated that the cross-sectional area of the intima and adventitia and its subcomponents were similar in adult SHR and WKY. With the exception of the preglomerular arterioles, all the renal arteries of adult SHR exhibited elevated cross-sectional quantities of total media, medial smooth muscle cells (SMCs), and extracellular space. Analysis of the SMCs indicated the presence of increased numbers of SMC layers and/or an increase in the SMC volume-to-surface area ratio in arteries sampled from adult SHR. Vascular contraction produced by infusing norepinephrine, BaCl2, angiotensin II, or by stimulating the renal nerves elevated the RVR to a greater degree in adult SHR than in WKY. The sensitivity of the renal vasculature to the various contractile agents was similar in adult SHR and WKY. When compared with WKY, prehypertensive SHR also exhibited increased cross-sectional quantities of arterial media and elevated amplitudes of RVR change in response to norepinephrine and renal nerve stimulation. However, the vascular contractile sensitivity to norepinephrine was reduced. Our results indicate that renovascular wall thickening and the hypercontractile reactivity associated with such a change precedes hypertension in SHR. In prehypertensive SHR, elevations in RVR might be counterbalanced by a decreased norepinephrine sensitivity. An increase in the norepinephrine contractile sensitivity and further vascular thickening with age could elevate the RVR and establish hypertension. (Circulation Research 1988;63:518–533)

Many researchers have argued that in all forms of hypertension, renal function must be reset in order for hypertension to develop.1-3 As stated by Tobian,4 regardless of the degree of increase in peripheral vascular resistance in various vascular beds, unless the resistance in the renal vasculature is also increased, hypertension will not develop, but rather a natriuretic response at the kidney will normalize blood pressure. Consistent with this view, the renal vascular resistance (RVR) is increased in Wistar Kyoto spontaneously hypertensive rats (SHR),4-6 and occurs at a very early age. At 8 weeks of age, the RVR of conscious SHR is twice that of Wistar Kyoto normotensive control rats (WKY).6 This suggests that the resetting of the renal vasculature may be of primary importance in initiating high blood pressure in SHR.

In the present study, an attempt was made to determine whether vascular structural and reactivity alterations of the type and magnitude that might facilitate the elevation of the renal vascular resistance are present in SHR and whether such alterations occur at an age where they may be important in initiating hypertension development in this animal model of essential hypertension. The renal vasculatures of SHR during an established (21 weeks of age) and prehypertensive (4 weeks of age) phase of hypertension development were fixed at maximal relaxation and processed for light and electron microscopic examination. Morphometric measure-
ments were performed on blood vessels extending from the main renal artery to the preglomerular arteriole and compared with similar measurements obtained from the kidneys of age-matched WKY.

If vascular wall thickening results in vascular contractile hyper-reactivity, it might be expected that all contractile agents, regardless of their mode of action, will produce greater amplitudes of RVR change in the renal vasculature of SHR than WKY, so long as the contractile agents exhibit equal contractile sensitivity for the renal vasculatures of the two groups of animals. In view of this, pharmacological and physiological experiments were undertaken involving the isolated kidneys of 21-week-old SHR with established hypertension and 4- or 5-week-old prehypertensive SHR and age-matched WKY. The RVR at maximal relaxation and the alterations in RVR in response to contraction by NE and other agonists and in response to renal nerve stimulation in the presence and absence of cocaine were determined in both age groups of SHR and WKY. In addition, in adult SHR and WKY, receptor antagonists were used to determine the types of receptors involved in producing nerve-mediated contractile responses and the proportion of the response attributed to each receptor type.

The data presented in this report suggest that structural alterations of a nature that could lead to renal vascular contractile hyper-reactivity are present not only during established hypertension but also just prior to hypertension development in SHR. In young SHR, such changes could play an important role in initiating hypertension.

Materials and Methods

Male SHR and WKY at 4 weeks (prehypertensive) and 21 weeks (established hypertension) of age were taken from a colony that is maintained at McMaster University, Hamilton, Ontario, Canada. A tail cuff compression method was used to measure the systolic blood pressure of prehypertensive SHR and age-matched WKY. In addition, prior to sampling, the systolic and diastolic blood pressure of prehypertensive SHR and age-matched WKY was measured using a direct technique. The rats were anesthetized (50 mg/kg sodium pentobarbital i.p.), and the aorta at the junction of the femoral artery was catheterized. The systolic and diastolic pulse pressures were recorded with a Statham P23Db pressure transducer connected to a recorder.

Perfusion System

The abdominal cavity of the anesthetized animals was opened, and the adrenal artery of the left kidney, the renal artery of the right kidney, and the mesenteric arteries were ligated. A catheter was then inserted into the aorta distal to the renal-aortic junction of the left kidney, in a manner that ensured continuous blood flow through the kidney. The segment of the aorta proximal to the left renal artery was clamped, and the left kidney of the rat was perfused with oxygenated (5% CO₂, 95% O₂) Krebs solution containing 1.5% dextran. The renal vein was then severed allowing a free outflow of the perfusate. This enabled the perfusate to flow only through the left kidney. A second catheter connected to a pressure transducer (Statham P23Db, Gould, Cleveland, Ohio) from a T junction about 4 cm upstream on the perfusate catheter allowed the perfusion pressure to be recorded. The vascular resistance was calculated by dividing the infusion pressure of the Krebs (compensated for the catheter resistance) by the infusion flow.

In preliminary experiments, the RVR was studied at various perfusion rates under conditions in which the vascular bed was maximally relaxed with Krebs solution containing 1) sodium nitroprusside (10 mg/l), 2) isoproterenol (10 mg/l), or 3) EGTA (5 mM). None of these agents produced further relaxation as indicated by the presence of a drop in RVR, suggesting that the renal vasculature maximally dilates when it is perfused with Krebs.

Preparation of Kidney Tissues for Light and Electron Microscopy

The vascularity of the left kidney was perfusion fixed under dilated conditions. Krebs solution containing 1.5% dextran (23°C) was perfused through the left kidney at a constant flow rate of 0.082 ml/min in prehypertensive SHR and age-matched WKY and 0.82 ml/min in SHR with established hypertension and matched WKY. The kidney was then perfused for 40 minutes with a solution of 2.5% glutaraldehyde, 1.86% sucrose, 0.063 M PO₄ buffer (pH 7.4, 400 mosm), and subsequently for another 20 minutes with a 0.200 M PO₄ buffer (pH 7.4, 400 mosm). The main renal and central interlobar arteries, as well as cortical tissue containing arcuate, interlobular arteries, and preglomerular arterioles, were then dissected out of the kidney and processed for light and electron microscopy using a method outlined by Lee et al. This method of tissue processing has been shown to produce minimal volume alterations in isolated rat aortic vascular smooth muscle cells.

For light microscopic measurements, 1 μm thick sections of the renal arteries, embedded in Spurr's resin, were cut in cross sections, stained with 1% azure II-methylene blue dye in 1% sodium borate, and mounted on glass slides. Subsequently, thin sections (600–800 Å) of each artery cut in the same plane of section were stained with Reynold's stain and examined with a Philips EM 301 electron microscope (Mahwah, New Jersey). The micrographs indicated a lack of convolution of the internal elastic lamina, confirming that the vessels were fixed under relaxed conditions.

Morphometry

Depending on the artery's size, each artery was photographed at the light microscope level at ×25, ×100, ×200, or ×400 magnifications. In the case of
the main renal artery, additional photographs were taken of the vessel wall at \( \times 400 \) magnification. Transmission electron micrographs of the entire arterial intima plus the media, or a maximum of 10 random frames per artery, were taken at \( \times 550 \) magnification for interlobar arteries, and at \( \times 720 \) and \( \times 1000 \) magnification for cortical arteries. The adventitia of all arteries was photographed at \( \times 2000 \) magnification, 10 random frames being taken for each artery. Due to the large wall thickness of the main renal artery, the intima and media were photographed separately at \( \times 1300 \) and \( \times 550 \), respectively; 10 frames of each layer being taken. Electron micrographs of arterioles were photographed at \( \times 550 \), \( \times 720 \), or \( \times 1000 \) magnification depending on the size of the arteriole. All 35-mm photographs taken at the light and electron microscope level were printed on 8\( \times \)10\( \)" paper after a further ninefold enlargement. Micrographs of a slide micrometer and an etched carbon-coated grid were used to determine the final magnification of light and electron micrographs, respectively.

The cross-sectional area of the lumen, intima + media, and adventitia were determined from low magnification light micrographs of the entire artery using a multipoint grid. The grid, which covered an 8\( \times \)10\( \)" micrograph, contained 143 equally spaced test points.

The following formula was used to calculate the cross-sectional area of the intima + media, adventitia, and lumen from light micrographs and to compensate these dimensions for section angle

\[
Ac = \frac{\pi x A_T}{P_T} \times \frac{R_S}{R_L}
\]

where \( Ac \) is cross-sectional area of an arterial component, \( Pi \) is number of points hitting the arterial component, \( P_T \) is total number of test points on the grid, \( R_S \) is short radius of lumen, \( R_L \) is long radius of lumen, and \( A_T \) is total area covered by grid.

A multipurpose grid was used to calculate the cross-sectional area of the subcomponents of the intima, media, and adventitia. The design of such a grid is outlined by Weibel.\(^9\) The grid used was 19\( \times \)19.2 cm and had 84 test lines with 168 test points, with each test line measuring 1.58 cm in length. In the intima + media, two lines were drawn across the artery perpendicular to the internal elastic lamina. The volume fraction of the endothelium, subendothelial space, internal elastic lamina, medial extracellular space, smooth muscle cells (SMCs), and the external elastic lamina were calculated. Within the adventitia, the volume fractions of adventitial collagen, fibroblasts, axons, nerve sheaths, and fluid-filled space were determined. The following formula was used to calculate the volume fraction of each medial and adventitial component

\[
V_x = \frac{Pi}{P_T}
\]

where \( V_x \) is volume fraction of a subcomponent, \( Pi \) is points hitting an arterial subcomponent (i.e. SMC, internal elastic lamina), and \( P_T \) is total number of points hitting all the subcomponents of the adventitial or nonadventitial arterial wall.

The true cross-sectional area of the intima and media and the subcomponents of these areas were calculated by multiplying the volume fraction of the wall occupied by these layers by the true cross-sectional area of the wall (\( A_w \)) obtained from Equation 1, for example, \( V_m \) media \( \times A_w = \) cross-sectional area of the media.

Under certain conditions, the comparative alterations in the mean SMC volume to SMC surface area ratio (V/S) can be used as an indicator of the presence of SMC hypertrophy in medial cross-sections. If individual vascular SMCs are modelled in the form of two equal-sized cones joined at the base, with the base of each cone having a radius, \( R \), and a peak-to-peak length, \( L \), it will be observed that the V/S ratio of the structure will be directly related to the volume under conditions in which the volume is altered by alteration of \( R \). Likewise, at V/S ratios \( \leq 1.0 \), the V/S ratio will also be directly related to the volume under conditions where \( R \) and \( L \) both increase, and where \( R \) and \( L \) vary in opposite directions. However, if in the above model, the volume is altered in a manner in which \( R \) is maintained constant and volume is altered by changing \( L \), the V/S ratio of the structure will not be greatly altered. In summary, if the above bicone model approximates the shape of a SMC, at ratios \( \leq 1.0 \) an increase in the SMC V/S ratio between two conditions will reflect an increase in SMC volume produced by either an increase in the midsection radius of the SMC or by some combination of change in SMC radius and length that increases volume. On the other hand, a lack of change in the SMC V/S ratio between two conditions does not necessarily indicate that SMC volume has not changed because volume changes could still be produced by a change in SMC length that would not greatly alter the SMC V/S ratio.

To estimate the SMC V/S ratio, the multipurpose grid previously described was used on electron micrographs of the media, and the number of grid points and surface intersects with the medial SMCs were recorded. The grid was then rotated 90\(^\circ\), and the count was repeated a second time. The formula outlined below was used to calculate V/S:

\[
V/S = \frac{z \times \Pi_{SMC}}{4 \times \Pi_{SMC}}
\]

where \( z \) is the line length of the test grid at each magnification, \( \Pi_{SMC} \) is the points hitting SMCs in the micrograph, and \( \Pi_{SMC} \) is the intersections of the lines with the surface of SMCs.
SMCs within the arterial media are arranged in an ordered pattern and represent anisotropic structures. In view of this, the value of V/S obtained using the above formula will vary with section angle.10 Therefore, the SMC V/S ratio values obtained from the analysis of the SMC profiles at one section angle will differ from the true SMC V/S ratio. However, if the arterial SMCs of SHR and WKY are cut at the same section angle, the difference between the calculated and true V/S ratio will remain constant and a comparative analysis of the SMC V/S ratio between the renal arteries of SHR and WKY is still possible. To maintain a constant section angle, the arteries sampled from SHR and WKY were cut in a plane perpendicular to the axis of blood flow. It should be noted, however, that despite the above effort, it could still be possible that the SMCs of renal arteries of SHR and WKY have differing shapes and patterns of helical pitch. If such was the case, the calculated SMC V/S ratio could differ between SHR and WKY despite the fact that no actual differences in SMC volume existed. In this regard, recent studies involving mesenteric arterioles have failed to show any difference in SMC shape or helical pitch when two age groups of SHR and WKY were compared,11 which suggests that the above concerns do not apply. However, until this is conclusively proven to be the case in the renal vasculature, alterations in the SMC V/S ratio values presented within this paper should be interpreted as suggesting, but not conclusively proving, the presence of altered SMC volume.

The number of medial SMC layers were counted in four quadrants spaced equally along the arterial wall using phase contrast light microscopy.

**Pharmacological and Electrical Stimulation**

**Studies Involving the Renal Vasculature**

All the dose-response curves involving contractile agents were performed using the perfusion system previously outlined. The temperature of the kidney and the perfusate were kept at 37°C by performing the experiment with a temperature-controlled Plexiglas case. A constant flow rate of 0.82 ml/min was used. At this flow rate, maximal contraction of the vasculature with norepinephrine (NE) or BaCl₂ produced the largest amplitude of infusion pressure change that could be linearly recorded by the pressure transducer (300 mm Hg upper limit).

**Dose Response Curves**

Cumulative dose response curves were obtained by infusing NE (0–10⁻³ M), K⁺ (0–100 mM), and BaCl₂ (0–48.7 mM) into the renal vasculature. With the exception of BaCl₂, the above chemicals were dissolved in Krebs solution containing 1.5% dextran aerated with 5% CO₂-95% O₂. Unless stated otherwise, separate groups of animals were used to study the different contractile agents. In studies involving NE, 10⁻⁶ M cocaine was included in the Krebs solution to prevent the uptake of NE by the periarterial nerves. In preliminary experiments, it was observed that contractile responses to 10⁻⁵ M NE were unaffected by 10⁻⁷ M propranolol (a β-receptor antagonist) and slightly reduced by higher doses of propranolol (>10⁻⁶ M). Therefore, it appears that doses of NE up to 10⁻⁵ M do not produce a significant level of β-receptor mediated relaxation. In view of this, the β-receptors were not blocked in experiments involving NE infusion. In studies involving K⁺ and BaCl₂ infusion, the experiments were performed in the presence of 10⁻⁸ M prazosin plus 10⁻⁶ M spiroperidol. These two agents, an α₁- and dopamine-receptor antagonist when used in combination, abolish the contractile response produced by supramaximal nerve stimulation (discussed in a later section); thus, the postsynaptic effects of any transmitters released from the nerves during the infusion of K⁺ or BaCl₂ were eliminated. In the case of BaCl₂ infusates, SO₄²⁻, PO₄³⁻, and HCO₃⁻ were omitted from the Krebs solution and the solution was buffered to pH 7.4 with 25 mM 3-[N-morpholino]propanesulfonic acid (MOPS). This adjustment was necessary to prevent BaCl₂ from forming a precipitate in the solution. This latter solution was aerated with 100% O₂. The osmolarity of the Krebs solution containing K⁺ or BaCl₂, was measured and maintained constant by decreasing the sodium chloride content in the Krebs solution in proportion to the level of K⁺ or BaCl₂ present.

The maximal amplitude of response to angiotensin II (Ang II) was obtained by infusing 10⁻⁷ M Ang II dissolved in Krebs containing 10⁻⁸ M prazosin plus 10⁻⁴ M spiroperidol through the renal vasculature. To test whether maximal response had been obtained, 10⁻⁶ M Ang II was infused after the 10⁻⁷ M dose. In all cases, no further contraction occurred.

In experiments involving other animals, platinum electrodes (subdermal; Grass Instruments, Quincy, Massachusetts) were placed in a jig that maintained the interelectrode distance at 2.5 mm. These electrodes were then placed around the main renal artery as it entered the hilum of the kidney. Field stimulation of the renal sympathetic nerves in this region resulted in vascular contraction and an elevation in the RVR. The renal nerves were stimulated with a potential difference of 60 V using 2-minute trains of pulses, each pulse having a 2-msec duration, at frequencies ranging from 2 to 14 Hz with a Grass S48 stimulator coupled to stimulus isolation unit. Maximum levels of RVR that could be obtained in response to nerve stimulation were usually achieved at stimulation frequencies of 10 Hz. Responses to nerve stimulation were totally abolished by a 10-minute infusion of Krebs containing 1 µg/ml tetrodotoxin, indicating that contraction was being mediated neuronally. Nerve stimulation experiments were carried out under normal conditions and under conditions where the neuronal reuptake of NE was blocked by the presence of 10⁻⁶ M cocaine. Response curves of RVR as a function of stimulation frequency were plotted.
To determine the receptors involved in producing nerve stimulated contractile responses, the nerves entering the kidney were field stimulated and the alterations in RVR were noted. The following receptor antagonists were then tested: 1) $10^{-7}$ and $10^{-6}$ M propranolol (a $\beta$-receptor antagonist), 2) $10^{-8}$ M prazosin (an $\alpha_1$-antagonist), 3) $10^{-7}$ and $10^{-6}$ M yohimbine (an $\alpha_2$-antagonist), 4) $10^{-7}$ M phenotamine (an $\alpha_1$- and $\alpha_2$-antagonist), 5) $10^{-6}$ M methysergide (a 5-HT receptor antagonist), and 6) $10^{-6}$ M spiroperidol (a dopamine receptor antagonist). Only $10^{-8}$ M prazosin plus $10^{-6}$ M spiroperidol totally abolished all nerve mediated responses. The fraction of the maximal nerve-mediated response attributed to $\alpha_1$- and dopamine receptors were calculated.

At the end of an experiment, edema formation was determined by comparing the wet weight of the perfused left kidney with that of the unperfused right kidney. No significant differences in wet weight between the perfused and unperfused kidney were observed in the experiments outlined above, which suggests that edema formation was minimal.

**Statistical Analysis**

An unpaired Student's $t$ test was used to analyze the statistical differences. The means of two groups were considered significantly different at $p<0.05$. All results were presented as the mean±SEM.

**Results**

**Morphometric Analysis of Renal Vasculature of SHR With Established Hypertension**

The average age and weight of the SHR ($n=6$) and WKY ($n=10$) used in the morphometric study was $21.3\pm0.2$ versus $21.3\pm0.4$ weeks and $352\pm15$ versus $334\pm3$ g, respectively. The systolic blood pressure of SHR was elevated over WKY ($194\pm4$ versus $121\pm2$ mm Hg, $p<0.005$).

**Lumen Diameter of Renal Vessels**

Table 1 outlines the lumen diameter of the renal vessels fixed in a relaxed state. SHR and WKY in an established phase of hypertension (21 weeks old) exhibited no significant difference in the lumen diameter of the main renal, interlobar, cortical arteries (arcuate-interlobular), or the preglomerular arterioles. In order to further investigate if the mean lumen diameter of the renal vasculature was altered in SHR, the isolated kidneys of another group of 21-week-old SHR and WKY, not used in the morphometric study were perfused under conditions where the renal vasculature was maximally relaxed. As shown in Figure 1, no significant difference in RVR was observed between SHR and WKY at perfusion/flow rates of 0.41, 0.82, 2.04 and 4.10 ml/min.

**Morphometric Analysis of the Renal Vascular Wall**

The structural alterations in the wall components are summarized in Tables 2 and 3. For

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**TABLE 1. Lumen Diameters (μm) of Various Classes of Renal Arteries Sampled From Adult (21-week-old) and Prehypertensive (4-week-old) SHR and Age-Matched WKY**

<table>
<thead>
<tr>
<th></th>
<th>SHR Main renal</th>
<th>SHR Interlobar</th>
<th>SHR Cortical arteries</th>
<th>SHR Arterioles</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-week-old</td>
<td>612±21</td>
<td>280±23</td>
<td>77±4</td>
<td>19±1</td>
</tr>
<tr>
<td>(6,6)*</td>
<td>(5,5)</td>
<td>(6,115)</td>
<td>(5,11)</td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>568±17</td>
<td>254±14</td>
<td>72±4</td>
<td>15±3</td>
</tr>
<tr>
<td>(5,5)</td>
<td>(5,5)</td>
<td>(8,121)</td>
<td>(5,13)</td>
<td></td>
</tr>
<tr>
<td>4-week-old</td>
<td>164±11</td>
<td>150±10</td>
<td>42±4</td>
<td>...</td>
</tr>
<tr>
<td>(9,9)</td>
<td>(9,15)</td>
<td>(10,104)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>155±12</td>
<td>149±5</td>
<td>39±2</td>
<td>...</td>
</tr>
<tr>
<td>(8,8)</td>
<td>(10,16)</td>
<td>(10,65)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SHR, spontaneously hypertensive rats; WKY, Wistar Kyoto rats.

*Sample size (rats, arteries).
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arteries studied (Table 2). Table 3 outlines the volume fractions of the adventitial subcomponents. In SHR, the volume fraction of fibroblasts was elevated in the interlobar arteries and in cortical arteries with a DI less than 95 μm. The volume fraction of collagen, nerve axons, nerve sheath cells, and fluid filled space was not altered with hypertension.

Table 4 outlines the alterations in the calculated SMC V/S ratios and in the number of SMC layers present in the renal vasculatures of SHR and WKY. It can be observed that the interlobar as well as the larger cortical arteries (with a DI >70 μm) sampled from SHR exhibited SMCs having larger morphometrically calculated V/S ratios than comparable renal arteries sampled from WKY. The calculated SMC V/S ratios of the main renal arteries and of the renal arteries/arterioles having a DI <70 μm were comparable between SHR and WKY. In SHR, the main renal arteries, cortical arteries with a DI >95 μm as well as prearteriolar arteries having a DI <70 μm exhibited greater numbers of SMC layers within the media when compared to comparable arteries sampled from WKY, but no significant differences in the numbers of SMC layers were observed in the interlobar arteries and in cortical arteries having a DI between 70–95 μm.
Morphometric Analysis of Renal Vascular Wall

The CSA of the various wall components of renal vessels sampled from prehypertensive SHR and age-matched WKY are shown in Table 5. The CSA area of the intima and of the media in the main renal artery and arcuate-interlobular arteries having a DI between 40 and 80 µm was increased in SHR. The CSA adventitia was increased in arcuate-interlobular arteries with DI between 40 and 60 µm in prehypertensive SHR. When the combined CSA of the intima and media was standardized to the CSA of the lumen (i.e., the ratio of the wall [intima + media] to the lumen), this ratio was increased in all arterial groups of prehypertensive SHR when compared with WKY. The number of SMC layers was greater in prehypertensive SHR than in WKY in the interlobar arteries and in arcuate-interlobular arteries with a DI greater than 80 µm and less than 60 µm.

Alterations in Contractile Reactivity and Sensitivity of the Renovasculature in Response to NE, BaCl2, and K+ Contraction in SHR With Established Hypertension

Figures 2, 3, and 4 show the alterations in RVR in response to the infusion of NE, BaCl2, and KCl in SHR with established hypertension. At maximal dilation, the RVR was similar between SHR and WKY. In the case of studies involving the infusion of NE or BaCl2, contraction of the renal vasculature produced diverging curves, the RVR being higher in SHR over WKY animals at concentrations of NE ≥2 × 10⁻⁷ M and BaCl2 ≥19.5 mM.

Unlike the results obtained in the NE and BaCl2 contraction studies, there were no differences in the magnitude of the potassium chloride contractile
TABLE 4. Renal Arterial SMC Surface-to-Volume Ratios and Medial SMC Layers in Adult SHR and WKY

<table>
<thead>
<tr>
<th></th>
<th>Main renal artery</th>
<th>Interlobar arteries</th>
<th>Cortical arteries*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample size</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>(rats, arteries)</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>(6,6)</td>
<td>(5,5)</td>
<td>(5,12)</td>
</tr>
<tr>
<td>WKY</td>
<td>(5,5)</td>
<td>(5,5)</td>
<td>(5,10)</td>
</tr>
<tr>
<td><strong>SMC layers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>0.81 ± 0.05</td>
<td>1.25 ± 0.06</td>
<td>0.85 ± 0.02</td>
</tr>
<tr>
<td>WKY</td>
<td>0.78 ± 0.06</td>
<td>0.81 ± 0.04</td>
<td>0.77 ± 0.03</td>
</tr>
<tr>
<td><strong>SMC volume/surface ratio</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>p &lt; NS</td>
<td>NS</td>
<td>0.05</td>
</tr>
<tr>
<td>WKY</td>
<td>5.73 ± 0.56</td>
<td>4.48 ± 0.137</td>
<td>3.51 ± 0.09</td>
</tr>
</tbody>
</table>

*These vessels were divided into four categories based on lumen diameter (μm), as measured between the internal elastic lamina of the blood vessel (Di).

SMC, smooth muscle cell; SHR, spontaneously hypertensive rats; and WKY, Wistar Kyoto rats.

Alterations in RVR in Response to Nerve Stimulation in SHR With Established Hypertension

Figure 5 shows the stimulation frequency versus RVR response curves obtained for SHR and WKY. Over WKY [respectively, 322 ± 13 versus 238 ± 3 mm Hg/(ml/min), p<0.005]. To determine if the contractile sensitivity was altered, the dose-response curves previously presented were replotted. Individual responses (RVR) were expressed as a percentage of the maximal change in RVR. Subsequently, the curves were analyzed for a rightward or leftward shift. No significant differences in ED50 values were observed between SHR and WKY. When SHR were compared with WKY, the ED50 values for NE were, respectively, 1.77 ± 0.34 versus 1.16 ± 0.32 M x 10^-7, NS; for KCl, 4.75 ± 2.1 versus 45.0 ± 2.9 mM, NS; and for BaCl2, 5.75 ± 1.00 versus 4.25 ± 0.79 mM, NS.

Alterations in RVR in Response to Nerve Stimulation in SHR With Established Hypertension

Figure 5 shows the stimulation frequency versus RVR response curves obtained for SHR and WKY.

TABLE 5. Morphometric Analysis of Renal Arterial Subcomponents of Prehypertensive SHR and WKY

<table>
<thead>
<tr>
<th></th>
<th>Main renal arteries</th>
<th>Interlobar arteries</th>
<th>&gt;80 μm</th>
<th>Arcuate-interlobular arteries</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>(rats, arteries)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>(9,9)</td>
<td>(9,15)</td>
<td>(7,10)</td>
<td>(8,17)</td>
</tr>
<tr>
<td>WKY</td>
<td>(8,8)</td>
<td>(10,16)</td>
<td>(7,13)</td>
<td>(6,10)</td>
</tr>
<tr>
<td><strong>Intima (μm²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>4,417 ± 548</td>
<td>1,849 ± 196</td>
<td>754 ± 86</td>
<td>443 ± 28</td>
</tr>
<tr>
<td>WKY</td>
<td>2,892 ± 239</td>
<td>1,470 ± 144</td>
<td>632 ± 47</td>
<td>346 ± 48</td>
</tr>
<tr>
<td><strong>Media (μm²)</strong></td>
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</tr>
<tr>
<td>SHR</td>
<td>20,874 ± 1,717</td>
<td>7,047 ± 860</td>
<td>2,941 ± 259</td>
<td>1,722 ± 83</td>
</tr>
<tr>
<td>WKY</td>
<td>16,567 ± 1,581</td>
<td>5,919 ± 503</td>
<td>2,473 ± 165</td>
<td>1,415 ± 148</td>
</tr>
<tr>
<td><strong>Adventitia (μm²)</strong></td>
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<td></td>
</tr>
<tr>
<td>SHR</td>
<td>33,261 ± 5,705</td>
<td>6,537 ± 751</td>
<td>3,365 ± 215</td>
<td>2,073 ± 107</td>
</tr>
<tr>
<td>WKY</td>
<td>37,557 ± 8,061</td>
<td>9,610 ± 1,581</td>
<td>3,774 ± 390</td>
<td>2,119 ± 83</td>
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<tr>
<td><strong>Wall lumen</strong></td>
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<tr>
<td>SHR</td>
<td>0.462 ± 0.025</td>
<td>0.485 ± 0.015</td>
<td>0.555 ± 0.023</td>
<td>0.682 ± 0.021</td>
</tr>
<tr>
<td>WKY</td>
<td>0.380 ± 0.019</td>
<td>0.405 ± 0.015</td>
<td>0.488 ± 0.030</td>
<td>0.556 ± 0.035</td>
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<tr>
<td><strong>SMC layers</strong></td>
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<td></td>
</tr>
<tr>
<td>SHR</td>
<td>4.53 ± 0.17</td>
<td>3.75 ± 0.11</td>
<td>2.65 ± 0.14</td>
<td>2.06 ± 0.04</td>
</tr>
<tr>
<td>WKY</td>
<td>4.30 ± 0.18</td>
<td>3.01 ± 0.11</td>
<td>2.36 ± 0.08</td>
<td>1.88 ± 0.15</td>
</tr>
</tbody>
</table>

SHR, spontaneously hypertensive rats; WKY, Wistar Kyoto rats; and SMC, smooth muscle cell.
FIGURE 2. Alterations in renal vascular resistance in response to cumulative doses of norepinephrine in isolated perfused kidneys of 21-week-old SHR (n=6) and age-matched WKY (n=7, mean±SEM). The experiment was performed in the presence of $10^{-5}$ M cocaine. Blood pressure of SHR compared with WKY: 190±5 vs. 122±4 mm Hg, $p<0.005$.

Under conditions in which the renal vasculature was perfused with Krebs in the absence of cocaine, the basal RVR was similar between SHR and WKY. When the nerves entering the kidney were stimulated at increasing frequencies, RVR was increased at frequencies greater than 6 Hz in SHR over WKY.

In a subpopulation of animals (four SHR and four WKY), the neuronal uptake of NE was blocked by the use of $10^{-3}$ M cocaine (Figure 5, right panel). The typical effect of cocaine was to increase the basal RVR and slightly shift the RVR response curve to the left of the curve obtained in the absence of cocaine. In the presence of cocaine, the basal RVR was the same in SHR and WKY while the amplitude of the response was significantly elevated in SHR over WKY at frequencies greater than 6 Hz.

Figure 5 suggests that the RVR at maximal levels of stimulation was greater in the presence of cocaine than in its absence. However, the subpopulation of rats used in the cocaine study (Figure 4, right) had slightly higher RVR at maximal stimulation than the overall group (Figure 4, left) from which the sample was taken.

To determine whether cocaine potentiated the response in SHR and WKY, the RVR at each frequency in the presence of cocaine was divided by the RVR at the same frequency in the absence of cocaine for each animal. Such analysis indicated that at lower stimulation frequencies of 2 and 4 Hz, cocaine potentiated the contractile response (twofold to fourfold) in both SHR and WKY, whereas at higher frequencies of stimulation (>4 Hz), cocaine had little effect in altering the RVR in response to electrical stimulation. Quantitatively, there was no significant difference in the degree of potentiation observed in SHR when compared with WKY.

Several receptor antagonists were used to assess the nerve-stimulated response (see "Materials and Methods"). These experiments were performed using four, 23-week-old SHR and four age-matched WKY (blood pressure of SHR compared with WKY: 171±1 versus 123±6 mm Hg, $p<0.05$). Initially, the renal nerves were electrically stimulated with a 2-minute, 10-Hz train of pulses with each pulse having a 2-msec duration and a 60-V potential difference. The application of such a stimulus produced a greater change in RVR in SHR over WKY animals. The introduction of $10^{-7}$ M propranolol had no effect on the response in SHR or WKY, while $10^{-6}$ M propranolol either had no further effect or lowered the response in SHR. The introduction of $10^{-8}$ M prazosin, an $\alpha_1$-receptor blocker, reduced the response by 80–95%. After prazosin, the responses obtained for SHR and WKY were not significantly different. The small residual response...
that was left was not affected by yohimbine or phentolamine (10^{-6} M) but was further decreased by the dopamine-receptor antagonist spiroperidol (10^{-6} M). On the basis of these findings, it would appear that nerve stimulated contraction is produced mainly by \( \alpha_1 \)-receptors. The proportion of the maximal response attributed to \( \alpha_1 \)-receptors is not significantly different between SHR and WKY (90.4 \pm 2.7\% versus 84.9 \pm 2.7\%, respectively; NS).

### Alterations in Contractile Sensitivity and Reactivity of Renovasculature in Response to NE Contraction in Prehypertensive SHR

Figure 6 shows the RVR responses to perfused NE (in the presence of 10^{-6} M cocaine) that were observed in the isolated kidneys of prehypertensive SHR and age-matched WKY. During Krebs perfusion, the RVR did not differ between prehypertensive SHR and WKY and represents the fully relaxed response. When the renal vasculature was contracted with NE, the dose-response curve obtained for prehypertensive SHR was shifted to the right of the dose response curve obtained for WKY. Thus, at lower concentrations of NE, RVR in prehypertensive SHR was higher than in WKY. But at concentrations greater than 10^{-6} M NE, RVR in prehypertensive SHR was higher than in WKY.

The sensitivity of the renal vasculature to NE contraction was reduced in prehypertensive SHR when compared with WKY. Comparison of the proportion of the maximal response produced by each dose of NE indicated that WKY had a higher fractional response than SHR at 10^{-7}, 2 \times 10^{-7}, and 5 \times 10^{-7} M doses in NE. The ED_{90} values for NE contraction were increased in prehypertensive SHR over WKY. The mean ED_{90} for NE contraction (as M \times 10^{-7}) for SHR and WKY was respectively, 3.20 \pm 0.56 and 1.80 \pm 0.36 (p<0.05).

### Alterations in RVR in Response to Nerve Stimulation in Prehypertensive SHR

The nerves entering the left kidney were stimulated at frequencies of 2, 4, 6, 8, 10, 14, and 16 Hz through the use of a 2-minute train of pulses. The values for RVR at each frequency of stimulation in the absence of cocaine are shown in Figure 7 (left). In the absence of nerve stimulation, RVR did not differ between prehypertensive SHR and WKY.
FIGURE 4. Alterations in renal vascular resistance in response to cumulative doses of potassium chloride in the isolated perfused kidneys of 21-week-old SHR (n=6) and age-matched WKY (n=6, mean±SEM). Blood pressure of SHR compared with WKY: 183±5 vs. 132±4 mm Hg, p<0.005).

Although RVR was higher in SHR than WKY at a stimulus frequency greater than 4 Hz, this was not significant at p<0.05. Figure 7 (right) shows the values for RVR during nerve stimulation when neuronal uptake of NE was blocked with 10⁻⁶ M cocaine. The presence of cocaine increased the basal RVR slightly in both SHR and WKY and resulted in significantly higher RVR in SHR compared to WKY at a stimulation frequency greater than 8 Hz.

Discussion

Structural Alterations in Renal Vasculature of Prehypertensive and Established Hypertensive SHR

In the present study, prehypertensive SHR and SHR with established hypertension had renal vascular resistances (RVR) that were similar to that of WKY when studied under maximally relaxed conditions. Consistent with this observation, other studies involving renal vascular beds in SHR have also failed to observe a consistent reduction in lumen diameter of blood vessels. For example, Folkow et al,12 Collis and Vanhoutte,13 and Collis et al14 did not observe a difference in RVR when SHR with established or incipient hypertension were compared with WKY or normotensive Wistar controls (NCR) under maximally relaxed conditions. Göthberg and his colleagues15 also observed that when the isolated kidneys of SHR and NCR were perfused with Tyrode's solution containing 2% dextran at low flow rates (<5 ml/min), the RVR was similar in adult SHR and NCR. However, in these studies when the flow rate was increased, the RVR increased to a greater extent in NCR than SHR. This phenomena occurred only under conditions where the kidneys were perfused with a solution capable of eliciting glomerular filtration. It was suggested that the apparent difference in RVR was produced by the presence of higher tissue pressures in the kidneys of NCR than SHR due to the fact that glomerular filtration was greater and occurred at lower perfusion pressures in the kidneys of NCR than SHR.15,16 Consistent with the above hypothesis Göthberg et al15 observed that when the renal vasculatures were perfused with a nonfiltering perfusate (kerosene), the RVRs were modestly elevated in SHR over NCR at high flow rates. These experiments suggest that a slight decrease in the preglomerular renal vascular lumen diameter and an increase in the preglomerular to postglomerular resistance may exist in the kidneys of adult SHR over NCR.

The most consistent observation made in both young and adult SHR was an increase in the cross-sectional area of the arterial wall of prearteriolar blood vessels. In adult SHR, wall thickening was shown to be produced by an increase in the medial quantities of extracellular space and SMCs. An analysis of the SMCs also indicated that many classes of renal arteries, sampled from SHR when compared with those of WKY exhibited increased numbers of SMC layers. The presence of increased numbers of SMC layers does not conclusively prove the presence of SMC hyperplasia. It could very well be that in the renal vasculature of SHR the above change could be coupled with a large decrease in SMC length, altering the packing of the SMCs in a manner in which no changes in SMC numbers occur, despite the fact that the numbers of SMC layers increase. However, recent studies involving mesenteric arteries and arterioles have indicated that SMC length is not altered when arteries from SHR and WKY are compared.17 In view of this, there is some reason to believe that the increased numbers of SMC layers observed in the renal arteries of SHR are in fact produced by the presence of SMC hyperplasia. An increase in the numbers of SMC layers, irrespective as to whether hyperplasia is or is not present, could have important functional consequences. By increasing the outer diameter of the blood vessels such an alteration could increase vascular reactivity and elevate the total peripheral resistance and thus help maintain hypertension.12 In the present study, it was observed that the SMC V/S was increased in the intermediate sized arteries of the renal vasculature of SHR when compared with WKY. As discussed in "Materials and Methods," if the helical orientation or the shape of the SMCs within the renal arterial wall does not differ between SHR and WKY, increases in the SMC V/S ratio will reflect an increase in SMC volume. In this regard, scanning electron micro-
Scope studies of the SMCs of mesenteric arterioles in young and adult SHR and WKY have indicated that neither the SMC shape, nor the helical pitch are significantly altered when SHR and WKY are compared. In view of this, there is reason to believe that the above concerns may not apply to the renal vasculature of SHR and WKY. If this was the case, the elevations in SMC V/S ratio observed in the renal vasculature of SHR would suggest that in addition to the increased numbers of SMC layers, SMC hypertrophy is also present.

In SHR with established hypertension, significant alterations in wall thickening were less prevalent in smaller than in larger blood vessels of the kidney. This is contrary to the widespread belief that the arterial lumen diameter is structurally decreased in hypertension and that the arterioles in particular are affected and help maintain hypertension. However, other studies carried out on nonrenal vascular beds have made observations consistent with those of the present study. In relaxed mesenteric arteries that were compensated for sectioning angle, Lee et al observed that wall thickening in adult SHR was produced by an increase in the arterial SMC cross-sectional area with no change in lumen diameter and was present only in arteries having a lumen diameter greater than 60 μm. In vivo microscopic studies involving microvasculature of the mesoappendix, abdomen, cremaster muscle, and gracilis muscle have indicated that the lumen diameters of the precapillary arterioles are either unaltered or enlarged in SHR when compared with WKY. As in the present study, the above studies have also failed to demonstrate the presence of vessel wall hypertrophy in SHR in the two or three orders of arterioles that precede the capillaries.

Furthermore, in the mesoappendix vasculature, Henrich et al observed that in SHR, the degree of vessel wall hypertrophy decreases as the vascular bed is followed toward the capillaries. In summary, the presence of a thickened vascular wall and a reduced lumen diameter in the precapillary arterioles of SHR is not a universal observation. Prearteriolar structural alterations in the vasculature such as those observed in the present study could still exert an important effect on vascular resistance. The amount of vascular resistance supported by such arteries is significant; for example, in the cremaster muscle of SHR and WKY, approximately 65% of the precapillary pressure fall occurs in arteries with a lumen diameter greater than 100 μm. Thus, it might be expected that structural alterations in prearteriolar renal vessels such as those observed in the present study could potentially exert an important effect in elevating RVR. The above hypothesis is consistent with some observations made on the renal vasculature of SHR. Hsu et al observed that the lumen diameter of the preglomerular arterioles was reduced in SHR. However, when hydralazine was infused into the renal vasculature of 12-week-old SHR, the elevated RVR returned to normal, while the preglomerular arteriole lumen diameter remained unchanged and below normal levels. This would suggest that sites other than the preglomerular arterioles are responsible for the elevated RVR and that the presence of SMC tone, as opposed to a permanent fixed decrease in lumen size, is important in maintaining the elevated RVR.

In the present study, wall thickening in the renal vasculature of SHR was found to occur prior to hypertension development. Although a prehypertensive phase of hypertension development was
observed in the present study, there is some disagreement as to whether a prehypertensive stage exists in SHR. Some studies have suggested that SHR are born with hypertension. Others indicate a prehypertensive stage that extends from 15 days to 12 weeks after birth. The discrepancy among these studies is likely due to a number of factors including the different methods used for blood pressure measurement, the degree of inbreeding in each SHR colony, the environmental conditions under which the colony is kept, and the diet fed to the rats. All these factors could accelerate or delay the onset of hypertension in SHR.

The observations made on the renal vasculature of prehypertensive SHR with respect to wall thickening in the present study are consistent with those made on the mesenteric vascular bed by Lee. In the latter study, it was observed that larger mesenteric arteries sampled from 4-week-old prehypertensive SHR exhibited an increase in the medial cross-sectional area produced by an increase in SMC layers. Mulvany and Nyborg also observed that the medial thickness to lumen diameter ratio of small mesenteric arteries was increased in 4-week-old SHR prior to hypertension development. However, in contrast to the present study, such changes were produced by a decrease in the lumen diameter rather than an increase in the quantities of arterial media. Other studies involving the mesenteric, tail, and carotid arteries of SHR ranging in age from 12 hours to 6 weeks indicate that wall thickening occurs at a time close to the onset of hypertension. However, the latter studies differ from those of Lee, Mulvany and Nyborg, and the present study in that the blood pressure was already significantly elevated in SHR over WKY at the time vascular wall thickening was observed.

Even though the RVR was not elevated in SHR under maximally relaxed conditions, during prehypertensive and established hypertensive phases, the structural changes observed could play an important role in initiating and maintaining an elevated blood pressure. If both WKY and SHR blood vessels are contracted from the adventitia in a manner where 1) each SMC contracts a similar proportion of its length, and 2) if during contraction the CSA of the media remains constant, or is similarly altered in SHR and WKY, the thick-walled hypertensive vessel would occlude its lumen to a greater degree than the thinner walled WKY vessel. Since, in vivo, the sympathetic nervous system is constantly active and maintains vascular smooth muscle cell tone, even normal sympathetic activity in combination with blood-borne constrictor agents would produce a situation in which hypertensive animals exhibit an increased RVR.
FIGURE 7. Alterations in renal vascular resistance in response to renal nerve stimulation in 5-week-old prehypertensive SHR (n=7) and age-matched WKY (n=7) (mean±SEM) in the absence (left) and presence (right) of 10^-4 cocaine. Blood pressure of SHR compared with WKY: 114±4 vs. 103±6 mm Hg, NS.

Because the structural changes observed in the present study occurred prior to hypertension in SHR, they are probably not a secondary adaptation resulting from the presence of high blood pressure. This would suggest that wall thickening in SHR is genetically programmed or occurs secondarily as a result of some as yet unknown trophic influence on the renal vasculature. If these structural changes were to cause the blood vessels to hyper-react to neuronal and or blood-borne vasoconstricting agents, such changes could play an important role in elevating the RVR. In turn, an elevation in the preglomerular RVR with respect to the postglomerular RVR would necessitate an elevation in blood pressure in order for normal glomerular filtration to take place. Hence, the structural changes observed in prehypertensive SHR could play an important role in initiating hypertension in SHR. However, at the present time, there is disagreement as to whether the mechanisms responsible for altering RVR in SHR are structural or functional. In vivo studies by Arendshorst and Beierwaltes have indicated that RVR is increased in 12 week old SHR. However, when the BP of the renal blood flow was reduced to that of WKY (from 158 to 114 mm Hg) via an aortic clamp, the renal blood flow and the glomerular filtration rate remained constant, while the RVR decreased to normal. It was concluded that in vivo extrarenal vasoconstrictor substance or intrinsic structural abnormalities did not play a role in elevating RVR in SHR. DiBona and Rios, on the other hand, found differing results in volume expanded 15-week-old SHR. The RVR of SHR was found to be in excess of two times that of WKY, regardless of whether the mean renal arterial pressure was elevated (177 mm Hg) or reduced to normal (106 mm Hg) by aortic constriction. This latter study suggests that some pressure independent intrinsic renal alteration is responsible for the elevated RVR in SHR.

Alterations in Renal Vascular Contractile Sensitivity and Reactivity in Prehypertensive and Established Hypertensive SHR

If a thickening of the vascular wall is responsible for the contractile hyper-reactivity alterations in the renal vasculature of SHR, it might be expected that all contractile agents, regardless of their mode of contraction would produce an increase in reactivity in SHR over WKY. Consistent with the above hypothesis, the renal arteries of SHR with established hypertension exhibited an increased reactivity in response to NE, BaCl2, Ang II, and renal nerve stimulation. Similarly, other studies of the renal vasculature have demonstrated contractile hyper-reactivity in response to vasopressin in stroke-prone SHR and serotonin in SHR. It is unlikely that the elevated amplitude of RVR response observed in SHR during NE contraction is due to an altered efficiency or effectiveness of postsynaptic receptors, since qualitatively, the same response can be elicited by BaCl2 which contracts vascular SMCs via nonreceptor mechanisms, and by Ang II which constricts by acting through nonadrenergic receptors. Furthermore, ED50 values for NE contraction were unchanged, and the proportion of the nerve mediated contractile response attributable to receptors (90–95%) was similar in SHR and WKY.
The renal vasculature of adult SHR did not, however, exhibit hyper-contractile reactivity in response to K+ depolarization. An explanation for the above anomaly could reside in the mechanisms involved in K+ contraction. Unlike NE which can contract via pharmacomechanical coupling and release internal stores of Ca2+, K+ contraction involves the opening of potential sensitive Ca2+ channels. It could be possible that in the renal vasculature of SHR, alterations in the mechanisms involved in K+ contraction exist which counteract the potential hyper-reactivity produced by the presence of a thickened vascular wall.

Vanhoutte and his colleagues carried out one of the most detailed studies of renal vascular sensitivity and reactivity in SHR. NE contraction studies indicated that the renal vasculature of SHR was more reactive and, in contrast to the present study, more sensitive to NE contraction. However, in spite of the elevated responsiveness to NE, the alterations in RVR were similar in SHR and WKY when the renal nerves were field stimulated. It was observed that the neuronal uptake of NE was increased and the release of NE was decreased during nerve stimulation. Based on this observation, it was hypothesized that the increased responses produced by the presence of hyper-reactivity and sensitivity to NE were counter-balanced by the presence of decreased levels of NE in the synaptic cleft during nerve stimulation. It was suggested that in adult SHR, RVR is elevated by central increases in sympathetic traffic or by the presence of locally produced or circulating facilitators of the NE release process.

Ekas et al. have presented results that are contrary to those of Vanhoutte and consistent with those of the present study. In these studies, it was shown that the neuronal release of NE was increased and the reuptake of NE by the nerves was unaltered in the renal vasculature of 20-week-old SHR. Consistent with the present study, it was demonstrated that SHRs exhibit greater increases in RVR than WKY in response to renal nerve stimulation.

In prehypertensive SHR, as in the established hypertensive group, RVR under relaxed conditions was not different when SHR were compared with WKY and SHR exhibited a higher amplitude of RVR response at maximum levels of NE contraction and nerve stimulation in the presence of cocaine. The degree of alteration in the renal vascular reactivity in prehypertensive SHR was less than that observed in adult SHR. This is consistent with the observation that, when compared with WKY, prehypertensive SHR have a renal vascular wall that is thickened to a lesser degree than that present in adult SHR.

In other studies, involving 6-week-old SHR which were hypertensive with respect to WKY, Vanhoutte and his colleagues also observed that the RVR of the isolated kidney of SHR was not different from WKY when the vasculature was normally relaxed. Electrical stimulation of the renal nerves at frequencies of 6, 8, 10, and 12 Hz resulted in a higher degree of RVR response in SHR than in WKY. However, unlike the present study, the elevated amplitude of response was statistically significant both in the presence and absence of cocaine and the reactivity of the renal vasculature to NE contraction was not altered when SHR and WKY were compared. On the basis of these and other experiments, it was suggested that the hyper-reactivity of the renal vasculature in response to nerve stimulation in SHR could be due to an increased release of NE from the renal nerves. In the present study, the neuronal release of NE from the renal nerves during nerve stimulation may be augmented in prehypertensive SHR and may contribute in part to the hyper-reactivity observed. However, in view of the fact that in the presence of cocaine the renal vasculature of prehypertensive SHR hyper-reacts to maximal NE contraction when compared to WKY, it appears that postsynaptic alterations in the renal vasculature, such as wall thickening may also play an important role in promoting hyper-reactivity.

A comparison of the NE ED50 values of all the animal groups indicates that adult SHR as well as young and adult WKY have similar NE ED50 values (1.77±0.35, 1.80±0.36, 1.16±0.31, respectively, expressed as M x 10^{-7}; NS) whereas the NE ED50 values obtained for prehypertensive SHR are higher (i.e., a decrease in NE sensitivity) than the above groups (3.20±0.56 M x 10^{-7}; p<0.05). An argument can be made that the BP of prehypertensive SHR could be maintained normal in spite of the presence of a thickened vascular wall (which would produce a higher vascular reactivity) because 1) the structural changes, although present, are not of sufficient magnitude to dramatically influence vascular resistance in vivo, and 2) the elevated renovascular reactivity to NE, in prehypertensive SHR, is counter-balanced in part by a decrease in sensitivity to this agonist. During development, the structural alterations producing an increased reactivity would be augmented and the NE sensitivity of the vascular bed wall increased. This, as well as the increased renal nerve firing activity that occurs in SHR over WKY after 5 weeks of age, may produce a situation where the RVR and BP become elevated in SHR over WKY.

References
Smeda et al
Structural Alterations in SHR Renovasculature


KEY WORDS • spontaneously hypertensive rats • renal vasculature • prehypertension • medial thickening • arterial hyper-reactivity
Structural and reactivity alterations of the renal vasculature of spontaneously hypertensive rats prior to and during established hypertension.

J S Smeda, R M Lee and J B Forrest