Pressure-Flow Curves Reflect Arteriolar Responses in Perfused Rat Hindquarters

Russell L. Prewitt, Colleen K. Reilly, Donna H. Wang

Abstract Results from studies using pump-perfused rat hindquarters are consistent with increased wall-to-lumen ratios in resistance vessels of spontaneously hypertensive rats (SHR). However, in vivo measurements of cremaster arterioles have not shown increased wall-to-lumen ratios in SHR. To investigate this discrepancy, we studied three groups of male SHR and Wistar-Kyoto rats at 12 weeks of age. In the first two groups, the cremaster muscle was prepared to allow microscopic observation while the hindquarters were pump-perfused with increasing concentrations of norepinephrine in oxygenated Tyrode's solution. Both groups of SHR showed an increase in vasodilated resistance and elevated maximal vasoconstrictr response. In the first group, arterioles showed dose-dependent constriction that was greater in smaller arterioles but did not differ between hypertensive and normotensive rats. Vasodilated diameters of second-order arterioles were significantly smaller in the hypertensive rats. In the second group, servo-null pressures in the first-order arteriole showed that the microvessels contributed proportionally to the elevation in resistance in both SHR and normotensive rats. In the third group, first- and second-order arterioles were measured in vivo and histologically. Arteriolar diameters did not differ between SHR and normotensive rats with either method. In fixed sections the cross-sectional area of the media-intima was greater in the SHR. Therefore, data from the pump-perfused rat hindquarters accurately reflect vasoconstrictr responses of the arterioles, and in deference to in vivo measurements on arteriolar walls that include the adventitia, the increased response in the SHR can be explained by hypertrophy of the arteriolar medial-intimal area. (Hypertension. 1994;23:223-228.)

Key Words • blood pressure • vascular resistance • microcirculation • norepinephrine • vasoconstriction • hypertension, spontaneous • arterioles

Experiments with pump-perfused rat hindquarters have shown an increase in vasodilated vascular resistance and an increased maximal responsiveness to vasoconstrictors in the spontaneously hypertensive rat (SHR). Those data have been interpreted as an indication that hypertrophy of the media of the resistance vessels encroaches on the lumen, giving a mechanical advantage through an increase in the wall-to-lumen ratio. These structural changes have been readily demonstrated in arteries larger than 150 μm, which contribute little to vascular resistance, but in vivo measurements of arterioles in skeletal muscles have shown neither lumen reduction nor elevation of the wall-to-lumen ratio in the SHR.

The discrepancy between the perfusion studies and the direct measurements of arteriolar structure has two possible explanations. Either the measurements of the arteriolar dimensions are incorrect, or the pump-perfused hindquarter preparation does not measure the response of the true resistance vessels, the arterioles. The latter possibility could be explained by the relative hypoxic conditions of the skeletal muscle microvascular bed when supplied by red blood cell-free medium. Although the larger vessels will receive an adequate supply of oxygen, the responsiveness of the arterioles may be severely inhibited by the fall in oxygen tension and exposure to elevated levels of vasodilator metabolites. On the other hand, the maximal doses of norepinephrine with added BaCl2 will activate the large arteries to supraphysiological levels, thus masking a weak or absent response in the arterioles. Because the arteries of the SHR are clearly hypertrophied, the maximal response will also be greatly enhanced in these animals. The increase in minimal vascular resistance may be due to arteriolar rarefaction in the SHR, which will eliminate parallel conductance pathways.

The experiments described below were designed to determine which of these two possibilities was correct by measuring the diameter response of the arterioles during a pump-perfused hindquarter experiment. Servo-null pressure measurements in the feed arteriole made it possible to divide the resistance response above and below that point, and histological measurements of arteriolar wall area were obtained to corroborate the functional data.

Methods

Pump-Perfused Hindquarters

Animal protocols were approved by the institutional Animal Care and Use Committee. Age-matched SHR and Wistar-Kyoto (WKY) rats (Harlan Sprague Dawley Inc, Indianapolis, Ind) were used for all experiments. Seven SHR and five WKY rats were anesthetized with 100 mg/kg sodium thiopental. The tail artery was cannulated and blood pressure was determined with a P23ID pressure transducer (Statham, Oxnard, Calif). The cremaster muscle was surgically prepared for microscopic observation by the method of Baez9,10 and covered with gauze soaked with physiological salt solution. The hindquarters were prepared for pump perfusion following the procedure of Folkow et al.14 Through an abdominal incision the intestinal contents were ligated and removed. The abdominal aorta and vena cava were isolated and cannulated below the renal
arteries with PE-190 and PE-205 cannulas, respectively. A steel clamp was tightened with two bolts across the abdomen of the rat to isolate the upper body from the hindquarters. The cremaster was spread over a Plexiglas pedestal and covered with polyvinyl film. The cremaster was maintained at 34°C with warm water circulated through the base of the pedestal, and a servo-controlled heating mat maintained the hindquarters at 37°C. The hindquarters were pump-perfused (Masterflex, Cole-Farmer, Niles, Ill) at 10 mL/min per 100 g with Tyrode's solution containing 3% Ficoll and equilibrated with 95% O₂ and 5% CO₂ at a pH of 7.4. The solution was not recirculated. For setting of the flow rate, the weight of the isolated hindquarters was taken as 40% of the body weight as previously shown.¹⁴ The preparation was placed on the stage of an ACM microscope (Carl Zeiss Inc, Thornwood, NY), and the rat was killed with an intracardiac injection of pentobarbital and phenytoin.

Under low power (×10), the arterioles in the cremaster muscle could be observed; if they did not clear of blood rapidly, the preparation was not used. The arterioles were classified as feeding (1A), arcading (2A), transverse (3A), or terminal (4A) according to their position in the network. Their diameter was measured by video image shearing using a UD20 objective for the 1A and 2A arterioles and a UD40 for the 3A and 4A arterioles. The inside wall of the arteriole could be readily observed even in the absence of blood. Perfusion pressure was determined initially through the tail artery cannula, which had to be bent to one side of the cremaster pedestal. After loss of data from some animals, perfusion pressure was recorded simultaneously from the perfusion cannula. In the latter case the pressure was corrected for the pressure drop in the perfusion cannula, which was measured at the end of the experiment. Perfusion pressure and arteriolar diameter were continuously recorded on a strip-chart recorder. Bolus injections of 10 mmol/L papaverine were introduced into the perfusion line to ensure a completely vasodilated preparation.

Arterioles were selected in a semirandom manner for measurement; that is, any type may be first, but it was our goal to obtain one of each classification from each preparation. While the diameter was recorded, norepinephrine was introduced into the perfusion line with a syringe pump to give a final concentration ranging from 0.15 to 4.0 μg/mL in a stepwise manner. Each concentration was given for 2 minutes, a time more than sufficient to reach steady state. After 1 minute of the 4.0 μg/mL dose, a 1-mL bolus injection of 0.1 mol/L BaCl₂ was introduced into the perfusion line. One minute later the norepinephrine was discontinued, and 1 mL of 10 mmol/L papaverine was injected to return the preparation to maximal vasodilation. This was repeated on as many as four different arterioles. The constrictor response of the arterioles was expressed as a percent of the initial diameter.

Servo-Null Pressures

Seven SHR and seven WKY rats were prepared as described above for observation of the cremaster microcirculation in the pump-perfused hindquarters, except that the cremaster was covered with physiological salt solution instead of polyvinyl film. Micropipettes were pulled and filled with 2N NaCl. Their initial impedance was 12 to 14 Mil, and the tips were polished until the impedance was reduced to 3 to 4 MΩ. A model 4A servo-null pressure system (IPM, Inc, San Diego, Calif) was used to measure the pressure in 1A arterioles during perfusion with increasing concentrations of norepinephrine after the protocol described above. The successful penetration of an arteriole was determined by momentarily stopping the pump and observing the simultaneous dip in arterial and arteriolar pressures. Once the pressure began to rise during norepinephrine infusion, the continued presence of the tip in the lumen of the vessel was readily ascertained.

<table>
<thead>
<tr>
<th>Group and Strain</th>
<th>MABP, mm Hg</th>
<th>BW, g</th>
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</thead>
<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR (n=7)</td>
<td>154±6*</td>
<td>261±3*</td>
</tr>
<tr>
<td>WKY (n=4)</td>
<td>99±3</td>
<td>249±7</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR (n=7)</td>
<td>134±7*</td>
<td>299±7</td>
</tr>
<tr>
<td>WKY (n=7)</td>
<td>84±5</td>
<td>292±9</td>
</tr>
<tr>
<td><strong>Group 3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR (n=10)</td>
<td>166±4*</td>
<td>300±5</td>
</tr>
<tr>
<td>WKY (n=11)</td>
<td>90±3</td>
<td>269±8</td>
</tr>
</tbody>
</table>

MABP indicates mean arterial blood pressure; BW, body weight; SHR, spontaneously hypertensive rat; and WKY, Wistar-Kyoto rat.

*P<.002 compared with WKY rats.

Histological Determination of Wall Area

Ten SHR and 11 WKY rats were anesthetized with 100 mg/kg sodium thiopental. The carotid artery was cannulated for measurement of arterial pressure, and the cremaster muscle was prepared for microscopic observation. The internal diameters of the 1A and 2A arterioles were measured in vivo while they were maximally dilated with topically applied 10⁻⁴ mol/L adenosine. After these measurements, the abdomen was opened, the animal was heparinized, the abdominal aorta cannulated, and 2 to 4 mL of blood was withdrawn. The cremaster was cleared and vasodilated by perfusion at the mean blood pressure of the rat with saline containing 10⁻⁴ mol/L adenosine, verapamil, and sodium nitroprusside. It was then perfusion-fixed with 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 mol/L cacodylate buffer, pH 7.4, at the same pressure. The withdrawn blood was injected into the perfusion cannula to fill the arterioles, and the cremaster was placed in fixative overnight at 4°C. The same sections of the 1A and 2A arterioles that were measured in vivo were cut out and placed in cacodylate buffer. After embedding in epoxy resin, 1-µm-thick sections were cut, and the area of the media was measured with a JAVA video image analysis system (Jandel Scientific, Corte Madera, Calif). Five repeated measurements of the same arteriole gave a standard error of 1.6% for medial area and 0.6% for inside diameter. Oblique sections were recut.

Results

Table 1 shows the mean arterial blood pressure of the three rat groups used in these studies. Although five WKY rats were used in the first series of experiments, arterial pressure was obtained on only four. Fig 1 shows that the initial perfusion pressures at maximal vasodilation and the responses to applied vasoconstrictors were significantly greater in SHR than in WKY hindquarters. As mentioned in “Methods,” problems with the cannula in the tail artery prevented successful recording of the higher-concentration data points in four SHR. The diameters of all arterioles observed under vasodilated conditions are shown in Table 2. Only the 2A arterioles of the SHR were significantly smaller than those of the WKY rats. The constrictor responses of the arterioles, shown in Fig 2, were quite pronounced and greater in the smaller arterioles than the larger. The average maximal vasoconstriction was just over 30% for the 1A arterioles, near 50% for the 2A arterioles, 60% to 70%
arteriolar diameter between SHR and WKY arterioles. Some shrinkage occurred in the fixed sections. However, there was a significant increase in medial cross-sectional area in both 1A and 2A arterioles of the SHR. This produced an increase in wall thickness and wall-to-lumen ratio in the SHR.

Discussion

Results of these experiments confirm the interpretation by Folkow et al.\textsuperscript{14} of data obtained on SHR and WKY pump-perfused hindquarters as indicative of hypertrophy of the smooth muscle in resistance vessels. The arterial perfusion pressures obtained with increasing concentrations of norepinephrine (Figs 1 and 3) duplicate those of Folkow et al.\textsuperscript{11,14} and several other investigators.\textsuperscript{15-17} As shown before, the minimal resistance of the SHR was significantly higher than that of the WKY hindquarters, and the maximal constrictor response was greater. The relaxed 2A diameters in the perfused hindquarters were smaller in the SHR (Table 2), thus contributing to the increased maximal resistance. Arteriolar rarefaction may also contribute to the higher perfusion pressure in the SHR hindquarters but not the elevation in maximal response.\textsuperscript{18}

Results in Fig 2 showed that, rather than being unable to develop tone because of hypoxic conditions, the arterioles responded with a vigorous constriction in response to perfusion with norepinephrine. Indeed, the smaller the arteriole, the greater was the response. Expressed as a percent constriction, there were no statistically significant differences between SHR and WKY arterioles, but the SHR arterioles contributed proportionally to their greater response by virtue of their smaller initial diameter.

The servo-null pressure measurements show that the arterioles contributed an increasing amount to vascular resistance with increasing concentrations of norepinephrine (Figs 3 and 4). The contribution to vascular resistance beyond the first-order arteriole was between 40% and 50% at maximal vasodilation, and rose to 60% to 70% at the highest concentrations of norepinephrine. Such an increasing contribution is expected because published results show that resting tone is much higher in arterioles\textsuperscript{6} than in arteries.\textsuperscript{19} Thus, when tone is abolished, the resistance in the arteriolar bed is proportionally less. Fig 3 shows that the 1A pressure in the SHR was significantly higher than in the WKY arteriole, confirming that the arteriolar bed contributed to the greater response of the SHR arteriole. Fig 4 shows no difference between SHR and WKY arterioles when the pressure in the 1A arteriole is expressed as a percentage

| Table 2. Arteriolar Diameters in Pump-Perfused Hindquarters Vasodilated With Papaverine |
|--------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| SHR                                             | WKY             |                 |                 |                 |
| 1A: 95.4±6.9 (n=7)                               | 102±15 (n=9)    | 16.4±0.9        |                 |
| 2A: 61.5±5.0 (n=10)                              | 80.2±7.1 (n=8)  |                 |                 |
| 3A: 32.9±5.7 (n=8)                               | 33.6±3.2 (n=8)  |                 |                 |
| 4A: 15.4±1.2 (n=6)                               |                 |                 |                 |
| P .04                                            | .9              | .5              |                 |

1A indicates feeding arteriole; 2A, arcading arteriole; 3A, transverse arteriole; 4A, terminal arteriole; SHR, spontaneously hypertensive rat; and WKY, Wistar-Kyoto rat. Numbers in parentheses are number of arterioles. Table includes vessels not shown in Fig 2 because complete dose-response curves were not obtained for them.
Fig 2. Line graphs show change in arteriolar diameter as percentage of initial diameter in cremaster muscle of rat hindquarters pump perfused at a constant flow rate with increasing concentrations of norepinephrine (NE). WKY indicates Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; 1A, feeding arterioles; 2A, arcading arterioles; 3A, transverse arterioles; and 4A, terminal arterioles.

of aortic pressure, indicating that the arteriolar contribution in the SHR arteriole, in absolute terms, was proportionally greater than that of the WKY arteriole.

Intravital measurements of relaxed arteriolar diameters (Table 3) confirmed the often reported lack of structural lumen reduction in the SHR when measured in vivo, where distending pressures in the SHR arterioles are greater than those of WKY arterioles.6-10 If pressures were equalized, the diameters of the SHR arterioles would be less than those of WKY arterioles because of elastic recoil. This is seen in the measurements of relaxed arteriolar diameter obtained during the pump perfusion experiments (Table 2), during which the pressure differential between SHR and WKY arterioles was smaller. Internal arteriolar diameters are the same in SHR and WKY arterioles in histological

Fig 3. Left, Line graph shows rise in perfusion pressure with increasing concentrations of norepinephrine (NE) in rat hindquarters pump perfused with oxygenated Tyrode's solution at a constant flow rate. Initial perfusion pressure after papaverine was 20±2 mm Hg for Wistar-Kyoto (WKY) rats and 45±4 mm Hg for spontaneously hypertensive rats (SHR); P<.005. Right, Line graph shows pressure in first-order arteriole (1A) of the cremaster muscle in the same rat hindquarters as shown on the left.
sections, because the vessels were perfusion-fixed at their mean arterial blood pressure. The diameters were slightly smaller than in vivo, as expected after dehydration, but the lack of a difference between SHR and WKY arterioles confirms the intravital measurements.

Values for arteriolar medial area and media-to-radius ratios, however, are quite different from intravital measurements, which most often show no hypertrophy in skeletal muscle arterioles. The intravital measurements are expressed as total wall area, which includes the adventitia. If the medial area increased and the adventitial area decreased, the intravital measurements would show no difference. As an example, Baumback et al. have shown that hypertrophy in the cerebral circulation of the stroke-prone SHR is associated with a greater percentage of smooth muscle in the arteriolar wall, whereas the external diameter of these vessels actually decreased.

Results similar to those reported here have been found in the intestinal arterioles. In a detailed study using transmission and scanning electron microscopy, Miller et al. found hypertrophy of vascular smooth muscle cells in 17- to 19-week-old SHR intestinal arterioles of approximately 60 µm in diameter. This hypertrophy did not result in smaller lumens, and it was not seen in the smaller arterioles. However, measurements from histological sections of arteries and arterioles supplying the spinotrapezius did not reveal any hypertrophy beyond the axillary artery in 15- to 20-week-old SHR. Elevated ratios of the arteriolar wall to radius of the SHR in this study were attributed to elastic recoil because the vessels were fixed at identical pressures in SHR and WKY rats. According to Lee et al., large arterioles of the mesenteric circulation are hypertrophied in the SHR at 10 to 12 and 28 weeks, but similar to the present findings the lumens were not significantly different from those of the WKY rats at either age.

There is interest in determining the role of growth factors and a possible genetic predisposition for the hypertrophy in SHR vessels based on the concept that hypertrophy of the vascular wall may be the cause of hypertension. However, most of the data are consistent with the hypothesis of Folkow et al. that vascular wall hypertrophy is a response to the elevated pressure rather than a cause of it. The gradient in vascular wall hypertrophy, from well developed in arteries to insignificant in terminal arterioles, suggests pressure rather than a circulating factor as the dominant regulator of wall growth. In studies in which local arterial pressure is normalized in the SHR, vascular wall morphology and function are also normalized, as demonstrated in perfused hindquarters or isolated small arteries. An enhanced growth response of isolated vascular smooth muscle cells from SHR aorta suggests a genetic predisposition for hypertrophy, but a similarly enhanced response can be obtained from vascular smooth muscle cells isolated from deoxycorticosterone acetate–salt hypertensive rats. Elevated pressure may activate genetic transcription that continues in the isolated cell in the absence of environmental regulation.

The lumen size of a normal artery or arteriole is not inversely related to the thickness of the wall. Wall area will increase in response to elevations in pressure, but in most cases the lumen does not decrease. In general, the wall increases with lumen size, but reduction in blood flow through an artery or arteriole in the absence of a pressure change will reduce the lumen without altering the wall area in an adult animal. In an immature animal in which the vessels are still growing, the wall area will be reduced along with the lumen in response to a reduction in blood flow. When blood flow increases without a change in pressure, as seen in the mouse transgenic for the growth hormone gene, both the lumen and medial areas of the aorta, carotid artery, and mesenteric artery increase. Interestingly, the wall-

#### TABLE 3. Arteriolar Wall Characteristics

<table>
<thead>
<tr>
<th></th>
<th>SHR (n=7)</th>
<th>WKY (n=7)</th>
<th>P</th>
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<tbody>
<tr>
<td><strong>Relaxed diameter in vivo, µm</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1A</td>
<td>139±5</td>
<td>133±6</td>
<td>NS</td>
</tr>
<tr>
<td>2A</td>
<td>95±6</td>
<td>101±3</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Fixed diameter, µm</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1A</td>
<td>114±4</td>
<td>114±5</td>
<td>NS</td>
</tr>
<tr>
<td>2A</td>
<td>86±5</td>
<td>88±4</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Medial area, µm²</strong></td>
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<td></td>
</tr>
<tr>
<td>1A</td>
<td>1673±61</td>
<td>1151±37</td>
<td>.0001</td>
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<tr>
<td>2A</td>
<td>990±88</td>
<td>773±53</td>
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<td><strong>Media thickness, µm</strong></td>
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<tr>
<td>1A</td>
<td>4.49±0.11</td>
<td>3.16±0.11</td>
<td>.0001</td>
</tr>
<tr>
<td>2A</td>
<td>3.46±0.20</td>
<td>2.69±0.10</td>
<td>.004</td>
</tr>
<tr>
<td><strong>Media/radius</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1A</td>
<td>0.080±0.004</td>
<td>0.057±0.005</td>
<td>.003</td>
</tr>
<tr>
<td>2A</td>
<td>0.082±0.005</td>
<td>0.062±0.003</td>
<td>.004</td>
</tr>
</tbody>
</table>

SHR indicates spontaneously hypertensive rat; WKY, Wistar-Kyoto rat; 1A, feeding arteriole; and 2A, arcading arteriole. Relaxed diameter was measured in vivo during topical application of 10⁻⁴ mol/L adenosine; remaining measurements were made by video image analysis of fixed sections.
to-lumen ratio remains the same in the aorta and carotid artery of transgenic mice, but the lumen increases less than the media in the mesenteric artery, producing an increase in the wall-to-lumen ratio despite normal blood pressure.

A major conclusion from these morphological studies is that elevated resistance in SHR hindquarter skeletal muscle primarily is due to increased tone rather than structural lumen reduction. This is in agreement with many in vivo studies of the microcirculation showing a greater amount of active tone in the SHR and no structural reduction in the lumen at prevailing pressures. Second, results from the pump-perfused hindquarter preparation correctly estimate the response of resistance vessels under those conditions, but the conclusions on lumen reduction are not necessarily those that prevail in vivo.

Acknowledgments
This work was supported by a Grant-in-Aid from the Virginia Affiliate of the American Heart Association and grant HL-36551 from the National Institutes of Health, Bethesda, Md. We thank Mary Beth Thompson for secretarial assistance.

References
Pressure-flow curves reflect arteriolar responses in perfused rat hindquarters.
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*Hypertension*. 1994;23:223-228
doi: 10.1161/01.HYP.23.2.223

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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