Microvessel Changes in Hypertension Measured by *Griffonia simplicifolia* I Lectin

Andrew S. Greene, Julian H. Lombard, Allen W. Cowley Jr., and Fay M. Hansen-Smith

Commonly used methods for assessing reductions in microvascular density (rarefaction) in hypertension detect only perfused microvessels. In the present study, samples of cremaster and spinotrapezius muscles were taken from rats with chronic (4-week) reduced renal mass hypertension and normotensive sham-operated control rats, as well as from 12-week-old spontaneously hypertensive rats and their normotensive Wistar-Kyoto control strain. Mean arterial pressure was 149±8 mm Hg in the rats with reduced renal mass hypertension, 114±7 mm Hg in sham-operated rats, 177±9 mm Hg in spontaneously hypertensive rats, and 95±4 mm Hg in Wistar-Kyoto rats. Muscle samples were incubated with rhodamine-labeled *Griffonia simplicifolia* I lectin, which identifies both perfused and nonperfused microvessels. Microvascular density was assessed by counting intersections with a 20-μm grid. Microvessel density was significantly reduced in cremaster muscles of both spontaneously hypertensive and reduced renal mass hypertensive rats, and in the spinotrapezius muscle of spontaneously hypertensive rats, compared with their respective normotensive controls. Further studies in the reduced renal mass rats on low salt diets indicated that lectin binding was also decreased as salt intake was increased, independent of blood pressure. This change was not due to an alteration in lectin-binding affinity. These studies indicate that lectin binding can be a useful tool for assessing microvascular density that does not depend on the perfusion state of the vessels and that rarefaction due to hypertension is not evenly distributed in all vascular beds. These results also provide evidence that dietary salt intake alone can influence microvessel density, as measured by the lectin technique. (Hypertension 1990;15:779–783)

Considerable evidence has been accumulated to indicate that both changes in vessel caliper and vessel number can contribute to the increased vascular resistance in hypertension.1–5 The degree to which variations in microvascular density alone contribute to the increase in total peripheral resistance in hypertension has been debated.1–5,6 Several studies have suggested that decreases in microvascular density occur in skeletal muscle in hypertension,2–5,7 whereas others have shown no change.9,10 In most of these studies, microvessel density has been estimated either from intravital measurements or from injection methods. Both of these techniques, however, rely on the perfusion status of the vessel at the moment of observation. Studies in spontaneously hypertensive rats (SHR)9,10 and in one-kidney, one clip renal hypertensive rats indicate that active closure of arterioles precedes anatomic rarefaction.4 Finally, cremasteric arterioles of rats in the early phases of reduced renal mass (RRM) hypertension are actively constricted and exhibit active closure and a high degree of vaso-motion.11 In these situations, an accurate measure of true anatomic vascular density might be difficult due to active constriction and a high incidence of complete closure.

In this study, we have evaluated a histological method for the determination of microvessel density based on the specific binding of *Griffonia simplicifolia* I (GS-I) lectin to microvascular structures. GS-I lectin, or its B4 isomer, binds selectively to basement membranes of microvessels,12 allowing for excellent visualization of the microvasculature.13–15 The present studies used rhodamine-labeled GS-I lectin to stain muscle whole mounts, which were then evaluated using computer fluorescence microscopy.

The objective of these experiments was to compare the lectin method with previously obtained results using vascular filling techniques in SHR and RRM hypertension. These comparisons were then used to evaluate the degree of anatomic rarefaction observed in the RRM model of hypertension.
Methods

Renal Mass Reduction

Male Sprague-Dawley rats were subjected to a 75% reduction in renal mass by a two-stage surgical procedure as previously described. The rats were 6 weeks old and 180–190 g in weight at the time of the initial surgery. Age-matched normotensive control rats underwent a sham operation (SOC) in which the kidneys were exposed, cleared of perirenal fat, and returned to the abdominal cavity. The rats were randomly assigned to one of the experimental groups herein described.

Experimental Groups

Three to five days after the final reduction in renal mass, the rats in the RRM and SOC groups were placed on either a high salt rat chow containing 4% NaCl or a low salt chow containing 0.4% NaCl for 4 weeks. One group of normal age-matched rats was placed on standard (0.8% NaCl) rat chow and studied after 4 weeks to verify that structural changes in the rats in the RRM group were the result of the renal mass reduction–salt loading procedure. All rats were allowed to drink water ad libitum. SHR and normotensive Wistar-Kyoto (WKY) rats were purchased at 12 weeks of age from Harlan Labs (Madison, Wisconsin) and were studied within 1 week of their arrival.

Hemodynamic Measurements

Rats were anesthetized with sodium pentobarbital and placed on a heated table. A catheter was placed in the left carotid artery and connected to a pressure transducer (Statham P23AC, Statham Instr. Div., Gould Inc., Oxnard, California) for the measurement of blood pressure and heart rate.

Histochemical Studies

Immediately after measurement of hemodynamic parameters, samples of the cremaster and spinotrapezius muscles were removed with a trephine and immersed in 30 μg/ml rhodamine-labeled GS-I lectin for 30 minutes to define the microvascular bed. After exposure of the tissue to the lectin, the muscle was rinsed thoroughly in physiological salt solution (PSS) and mounted on a microscope slide with a water-soluble mountant. Samples were studied as whole mounts by using a video fluorescent microscope system with epi-illumination.

Figure 1 shows a photomicrograph of a cremaster muscle labeled with rhodamine GS-I lectin. Microvascular density was measured by counting the intersections of fluorescently labeled microvessels with a 20-μm computer-generated grid overlaying the microscope field observed at ×300. Vessels of order 3 and below were visible after lectin treatment. No attempt was made to classify vessels by order. Two slides of each muscle were studied. Five fields from each slide were randomly selected and counted. The results from the 10 fields were averaged to give a single density for each muscle.

Lectin-Binding Affinity

In a final series of experiments, muscle samples were removed from normal Sprague-Dawley rats to
evaluate the effect of sodium chloride concentration on lectin-binding affinity. The muscle was homogenized, and the homogenate was centrifuged. The supernatant was discarded, and the pellet was resuspended in PSS with sodium concentrations varying between 135 and 155 meq/L. GS-I lectin was then added to the suspension at a final concentration of 15 μg/ml and incubated for 30 minutes at room temperature. The suspension was centrifuged, and the supernatant was saved. The pellet was then washed with three rinses of PSS, and the suspension was returned to its initial volume. Rhodamine-lectin concentration in both the saved supernatant and the suspension was measured spectrophotometrically, and a free/bound ratio was computed.

### Statistical Analysis

All results were expressed as the mean±SEM. Differences between groups were analyzed by a two-factor analysis of variance with no repeated measures. Significant differences between individual means were determined using Duncan’s new multiple range test. Values of \( p < 0.05 \) were considered significant.

### Results

#### Hemodynamic Parameters

As previously reported, blood pressure was elevated both in rats in the RRM group on high salt diets\(^1\) and in SHR rats on normal chow. All other groups of rats had normal blood pressures. Heart rates were higher in rats on a high salt diet than the respective control rats on a low salt diet although an elevation in blood pressure did not occur in some of these groups. Heart rate and blood pressure for all groups of rats are summarized in Table 1.

#### Microvascular Density Changes

Microvascular density, as demonstrated by the lectin technique, was reduced in both the cremaster and spinotrapezius muscles of the SHR, relative to those of normotensive WKY control rats (Figure 2). Vessel density was reduced by 14.1% in SHR cremaster and 13.8% in SHR spinotrapezius muscles. Vessel density was reduced by 22.8% in the cremaster muscle of hypertensive rats in the salt-loaded RRM group, relative to the normotensive SOC rats on a high salt diet. Microvessel density in the spinotrapezius muscle, however, was not significantly different in the rats in the RRM and SOC groups on a high salt diet. Vessel densities in rats in the SOC group were not different from those measured in normal age-matched Sprague-Dawley rats fed normal rat chow. These results are summarized in Figure 3.

### Table 1. Hemodynamic Measurements

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP</th>
<th>HR</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>RRM 0.4%</td>
<td>104±13.7</td>
<td>314±20.3</td>
<td>7</td>
</tr>
<tr>
<td>RRM 4%</td>
<td>131±13.1</td>
<td>355±11.2</td>
<td>14</td>
</tr>
<tr>
<td>SOC 0.4%</td>
<td>105±4.2</td>
<td>303±10.1</td>
<td>7</td>
</tr>
<tr>
<td>SOC 4%</td>
<td>114±5.5</td>
<td>365±15.7</td>
<td>17</td>
</tr>
<tr>
<td>Normal 0.8%</td>
<td>108±9.9</td>
<td>337±19</td>
<td>9</td>
</tr>
<tr>
<td>SHR</td>
<td>177±8.9</td>
<td>351±9.9</td>
<td>17</td>
</tr>
<tr>
<td>WKY</td>
<td>95±4.12</td>
<td>295±12.3</td>
<td>17</td>
</tr>
</tbody>
</table>

Values are mean±SEM. MAP, mean arterial pressure; HR, heart rate; RRM, reduced renal mass; SOC, sham-operated control; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto; LS, low salt diet; HS, high salt diet.

*Indicates significantly different from LS control rats.
†Indicates significantly different from HS SOC rats.
‡Indicates significantly different from WKY rats.

### Figure 2. Bar graphs showing reduction of microvessel density in spinotrapezius muscle (upper panel) and cremaster muscle (lower panel) in spontaneously hypertensive rats (SHR) (open bars) and Wistar-Kyoto (WKY) rats (solid bars). *Indicates significant differences from WKY rats.

### Figure 3. Bar graphs showing microvessel density in spinotrapezius muscle (upper panel) and cremaster (lower panel) of hypertensive rats in the reduced renal mass (RRM) group (open bars) and normotensive sham-operated control (SOC) rats (solid bars). *Indicates significant differences from SOC rats.
Although the lectin technique demonstrated vessel rarefaction in both the SHR and the rats in the RRM group, salt intake also had a significant effect on the estimated vessel density, which was independent of blood pressure. Figure 4 summarizes the microvessel densities for rats in the SOC group on low and high salt diets. Salt intake alone decreased measured vessel densities by 16.5% in the cremaster muscle but had no effect on the spinotrapezius muscle.

Lectin-Binding Affinity

The reduction in measured vessel density in rats on a high salt diet was clearly not due to a direct effect of sodium chloride on lectin binding. Increasing sodium chloride concentration in five steps between 135 and 155 meq/l in muscle homogenates did not decrease the binding of the lectin molecule as measured by the free/bound ratio. Therefore, the affinity of lectin binding to microvascular structures in muscle homogenates was not affected by sodium chloride concentration per se.

Discussion

The techniques that have been previously used to measure microvascular rarefaction in hypertensive animals are limited because of their reliance on vessel perfusion to deliver a dye or a filling compound (India ink, silicone rubber compounds, or various resins) to the microcirculation. In hypertension, alterations in the vasculature can cause inhomogeneous tissue perfusion, increased vascular tone, excessive vasomotion, or vessel degeneration. Therefore, methods that depend on the perfusion status of the vessel might not be suitable for quantitation of vessel density.

The results of the present study indicate that the use of a specific marker such as the GS-I lectin molecule, which does not depend on the perfusion status of the vessel, is valuable when assessing microvascular rarefaction. With this technique we have demonstrated significant rarefaction in the cremaster muscle of hypertensive rats in the RRM group and in SHR. Previous studies using less specific techniques have measured rarefaction of a similar magnitude in cremaster muscles of SHR and RRM rats.

In the present study, we could not demonstrate rarefaction in the spinotrapezius muscle of rats in the RRM group. This heterogeneous distribution of rarefaction in the rats in the RRM model might be due to a redistribution of arteriolar resistance resulting from nonuniform closure of microvessels, which probably proceeds to the anatomic rarefaction. This nonuniformity could be due to differences in sympathetic innervation or local autoregulatory responses in these vascular beds.
binding was observed in vessels with diameters greater than 20 \( \mu m \).\(^{13}\)

In the present study, we observed a significant effect of sodium intake on the apparent vessel density in the cremaster muscle of both hypertensive rats in the RRM group and normotensive rats in the SOC group. Vessel density changes in the cremaster muscle of hypertensive rats in the RRM group are in good agreement with our previous studies using vascular filling techniques.\(^{16}\)

The present experiments suggest that a high salt diet alone might cause a loss of microvessels despite a normal blood pressure. One change that is known to occur when salt intake is elevated is a remodeling of collagen in the vascular basement membranes.\(^{17}\)

Our recent ultrastructural studies suggest that high salt intake might indeed have effects on the morphology of microvessels and the integrity of their basement membranes.\(^{15}\)

This loss of basement membrane integrity might be an early stage of vessel degradation that proceeds to anatomic rarefaction.

Another possibility that needs to be considered is that alterations in vascular basement membranes could lead to a loss of lectin-binding sites that would result in the decrease in apparent vessel density observed with high salt intake. Our in vitro studies on lectin binding did not show any dependence of lectin-binding affinity on sodium concentration. Additionally, comparison studies using both the GS-I lectin and alkaline phosphatase methods have shown that GS-I lectin reveals significantly more capillaries in animals on normal salt diets\(^{13}\) and that the binding of the GS-I molecule is less affected by age and capillary neof ormation activity in adult muscle than the alkaline phosphatase method.\(^{14}\)

Thus, it appears that the specific lectin binding to microvessels is quite stable under a variety of physiological conditions.

Although the measured vessel density in the cremaster muscle of rats in the RRM and SOC groups was influenced by the sodium intake, in these experiments, this effect was apparently additive to the effect of an increase in blood pressure. Figure 5 summarizes our data and indicates that the effect of salt, like the rarefaction itself, appears to be confined to the cremaster muscle and suggests that both a high salt diet and hypertension contribute to the loss of blood vessels in the rats in the RRM group.

We have used the GS-I lectin technique to demonstrate microvessel rarefaction equivalent to that reported in other studies.\(^{7}\)

We have confirmed the presence of rarefaction in rats with chronic hypertension both with vascular filling\(^{16}\) and ultrastructural studies.\(^{15}\)

Additionally, SHR and WKY rats on normal salt chow exhibited large differences in vessel density by the lectin technique. This reduction of vessel density demonstrated by the lectin technique in SHR was independent of salt intake. Thus, it appears that the GS-I lectin technique allows a specific and perfusion-independent assessment of changes in the microvasculature that result from hypertension and might provide a valuable tool in understanding the early changes in microvessel morphology that lead to rarefaction.

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References


**Key Words**

- blood pressure
- blood flow
- microcirculation
- histology
- fluorescent indicators
- vascular tissue
- capillaries
- rarefaction
- reduced renal mass hypertension
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