Longitudinal Study of the Hindquarter Vasculature During Development in Spontaneously Hypertensive and Dahl Salt-Sensitive Rats

SHIRLEY M. MUELLER, M.D.

SUMMARY The purpose of this study was to examine vascular structural alterations longitudinally in spontaneous and Dahl genetic hypertension. Hypertensive and control animals were studied at 5, 9–11, and 17–19 weeks of age to permit analysis of prehypertensive, early and established hypertensive stages. Minimal and maximal resistance of the hindquarter vasculature was used as a functional assessment of structural alterations. At 5 weeks of age, the minimal vascular resistance of the spontaneously hypertensive rats (SHR) was elevated over Wistar-Kyoto (WKY) \((p < 0.02)\) but there was no difference between Dahl salt-sensitive (S) and resistant (R) rats. In both sets of animals, the minimal vascular resistance of the hypertensive group was significantly elevated over controls with age: \(p < 0.001\) in SHR; \(p < 0.001\) in Dahl S. The maximal vasoconstrictor response was significantly greater with age in SHR than in WKY, \((p < 0.001)\), but was not different in Dahl S compared to R. Thus, structural alterations, determined by assessing minimal vascular resistance, are present in both spontaneous and Dahl salt-sensitive hypertension, but the origin of the two differ. An increase in smooth muscle mass, assessed by maximal constriction, contributes importantly to the structural alterations in spontaneous hypertension; in Dahl S, other factors appear to contribute to structural alterations. Further, structural alterations precede frank hypertension in SHR but not in Dahl S hypertension. (Hypertension 5: 489–497, 1983)

KEY WORDS • genetic hypertension • hindquarter • longitudinal • vascular changes

ELEVATED peripheral resistance is found in association with hypertension. Among factors thought to contribute to an elevated peripheral resistance is a structural alteration in the hypertensive vascular bed. The origin of the vascular alteration is uncertain. Folkow and colleagues\(^1\)\(^-\)\(^2\) attributed it to an elevated blood pressure which stimulates vascular hypertrophy through an increased stress on the vessel wall. They demonstrated structural alterations in both spontaneous\(^1\) and renovascular\(^2\) hypertension associated with an elevated blood pressure. Other groups suggested that structural alterations, consisting of vascular hypertrophy\(^3\)\(^-\)\(^4\) or a decreased number of arterioles,\(^5\)\(^-\)\(^6\) are present early in the spontaneously hypertensive rats (SHR) and thus cannot solely be explained by blood pressure elevation. Dahl salt-sensitive (S) and resistant (R) genetic hypertension have not been similarly studied during early hypertension.

The purpose of this study was to examine hindquarter vascular structural alterations longitudinally in spontaneous and Dahl genetic hypertension. Spontaneously hypertensive rats (SHR) and their controls, Wistar-Kyoto (WKY), as well as Dahl S and R rats were examined. The animals were divided into three groups: prehypertensive (5 weeks old), early hypertension (9 and 11 weeks old) and established hypertension (17 and 19 weeks old). Minimal and maximal resistance of the hindquarter vasculature was used as functional assessments of structural alterations.\(^7\) Minimal vascular resistance reflects the cross-sectional area of the resistance vessels at complete relaxation. A structural change of either a passive or active component of the vessel wall that causes impingement on the vessel lumen at maximal dilation would alter minimal vascular resistance.\(^8\) On the other hand, the active smooth muscle contractile contribution to resistance is reflected in the maximal constrictor response since an increase in passive wall elements would only slightly increase maximal constriction.\(^9\) Thus, these studies were designed to assess for the presence of vascular structural alterations (minimal vascular resistance) and their possible progression with age and blood pressure elevations (different ages studied) as well as the contribution
of smooth muscle to any potential structural alterations (maximal constriction). In addition, vascular reactivity to norepinephrine was simultaneously studied.

Methods

Animals
A total of 30 SHR and 30 WKY male rats (Taconic Farms, Germantown, New York) as well as 27 S and 26 R Dahl female rats (Brookhaven National Laboratory, Upton, New York) were examined. The rats were maintained in animal rooms apart from other laboratory animals. The SHR and WKY were 5, 9, and 19 weeks old when studied. The Dahl S and R were 5, 11, and 17 weeks old when examined. The SHR and WKY were fed standard laboratory rat chow (0.4% sodium, 1% potassium). The Dahl rats were placed on either a sodium deficient (5-week group) or high sodium diet (11- and 17-week groups). These special diets were obtained from ICN Nutritional Biochemicals (Cleveland, Ohio). The sodium-deficient diet, rat-modified, contained a sodium-free salt mixture and 2% potassium salt. The high sodium diet contained 8.98% NaCl and 1% potassium salt. The 5-week-old Dahl rats were on a sodium-deficient diet for 2 weeks after weaning and then studied. The 11- and 17-week-old Dahl rats were on a high sodium diet from weaning and 14 weeks respectively and then studied.

Hindquarter Perfusion
The rats were anesthetized with pentobarbital sodium (40 mg/kg) intraperitoneally. The carotid artery was catheterized in the 5-week-old animals, and the brachial artery was catheterized in the older animals for measurement of blood pressure. Heparin (100 μl/kg) were injected into the brachial artery for anticoagulation. A tracheal cannula was inserted to facilitate spontaneous respiration during surgery.

Hindquarter perfusion pressure was determined according to the method described by Folkow using an artificial solution, Tyrode solution with Ficoll. The abdominal aorta was exposed through a midline incision. Skin, muscle and peritoneum were retracted, and the intestines packed to one side with a moist piece of cotton gauze. The aorta and vena cava were ligated below the kidneys. The distal descending aorta was cannulated above its bifurcation with PE-50 tubing that was connected via a t-tube to a Harvard infusion pump and a Statham pressure transducer. Pressure was recorded on a Gould recorder, model 2400. The distal vena cava was opened widely just as the perfusion was started to facilitate egress of the artificial perfusate. The perfusate consisted of Tyrode's solution (g/liter: NaCl 8.0; KCl 0.2; CaCl2·2H2O 0.2; MgCl2·6H2O 0.1; NaHCO3 1.0; NaH, PO4·H2O 0.05 and glucose 1.0) containing a plasma expander, Ficoll 70, mol wt 70,000, 3% weight per volume (Pharmacia Fine Chemicals, Piscataway, New Jersey). The solution was saturated with 95% oxygen and 5% CO2 to attain physiological pH and pCO2, and then bubbled with oxygen to maintain pO2 > 400 mm Hg. The osmolality was between 290 and 300 milliosmols. Before reaching the animal, the perfusate was warmed to 37°C. A 37°C water-heated pad was placed under the hindquarter. From preliminary experiments, hindquarter weights were determined to be 30% of the total body weight in 5-week-old animals and 34% of body weight in animals 9 weeks and older. The flow rate of the perfusate was 3.0 ml/min/100 grams hindquarter weight. The rat was killed by hemorrhage from an incision in the aorta proximal to the infusion catheter. Fifteen minutes later, the perfusion pressure was consistently stable.

Perfusion Pressure at Maximal Dilation
Before producing maximal dilation, neural influences on the hindquarter vasculature were eliminated by sectioning the cervical spinal cord. In preliminary experiments we found that sectioning the lumbar cord resulted in identical responses to those obtained when we sectioned the cervical cord. Papaverine, 0.04 mg/ml in Tyrode solution, was used to produce maximal dilation. We have established that this concentration of papaverine produces maximal dilation in two ways. First, higher concentrations did not cause a further decrease in perfusion pressure. Second, maximal dilation was independently confirmed by ceased perfusion of the hindquarter for 5 minutes and, at the end of this "ischemic" period, flow was started again and perfusion pressure was recorded. There was no difference between perfusion pressure obtained with papaverine and with "ischemic" arrest. Longer periods without perfusion produced no greater fall in resistance. Pressures were obtained at four different flows during maximal dilation. The hindquarter was then perfused with Tyrode solution containing Ficoll without papaverine at 3.0 ml/min/100 g hindquarter weight for about 15 minutes. During this time, the perfusion pressure returned to the previous baseline.

Norepinephrine Sensitivity
To assess norepinephrine sensitivity, a dose-response curve to low concentrations of norepinephrine was constructed by addition of norepinephrine to the perfusate. The flow rate of 3.0 ml/min/100 g hindquarter weight was continued. The norepinephrine concentrations began with 0.01 μg/ml and continued through 0.03, 0.10, 0.16 and 0.60, 1.0, and 10 μg/ml until a steady state response was achieved at each concentration.

Maximal Vasoconstrictor Response
Finally, to test maximal vasoconstrictor response, vasopressin (10 IU in 0.5 ml solution) followed by barium chloride (150 mg in 0.15 ml of 0.9% NaCl) were injected in sequence into the hindquarter perfusate. A maximal response with each was obtained before the second vasoconstrictor substance was added. The hindquarter of the rat was then weighed so the flow/100 g hindquarter weight could be calculated. The hindquarter weight was equal to the preexperimental total body weight minus the forequarter weight.
Calculation of Data and Statistics
At the end of the experiment the pressure generated from the resistance of the perfusion tubing was determined and subtracted from the recorded pressure to determine true perfusion pressure. A two-way analysis of variance was used to determine differences between the flow-pressure curves of the hypertensive and normotensive animals at maximal dilation and between the minimal and maximal vascular resistance at different ages. Vascular resistance was determined by dividing perfusion pressure (mm Hg) by flow (ml/min/100 g hindquarter). Norepinephrine sensitivity was analyzed by determining threshold sensitivity to norepinephrine, slope of the norepinephrine dose-response curve, and the concentration of norepinephrine that produced 50% of the maximal response to norepinephrine. ED50 Threshold was defined as that concentration of norepinephrine which was required to raise vascular resistance 25% above baseline, and compared between the two groups using an unpaired t-test. The slope of the norepinephrine dose-response curve was determined using the straight part of the norepinephrine (norepinephrine = 0.60, 1.0 and 10 µg/ml) dose-response curve and compared between the two groups using the unpaired t-test. The ED50 was likewise compared between the two groups using the unpaired t test.

Determination of Edema
We determined possible edema formation in two ways. One was by comparing the calculated hindquarter weights before and after the study. If fluid retention had not occurred secondary to edema, the weights should be identical. These weights did not differ in experiments in each group. The calculated hindquarter weight was determined by weighing the total rat prior to the experiment. After the experiment, the upper body cephalad to the hindquarter perfusion was weighed. Then the upper body weight was subtracted from total body weight to obtain calculated hindquarter weight. This weight was then compared to the actual hindquarter weight. The correlation of the recorded hindquarter weight and the hindquarter area being perfused was further assessed by infusion of Evan’s blue dye into the hindquarter at the end of the study. The hindquarter area stained with the dye correlated directly with the level of body sectioning for hindquarter weight.

The second method of determining edema formation was apparently more sensitive because changes indicative of edema were found with this method when they were not observed with the first. This method consisted of frequent determinations of baseline perfusion pressure at constant flow throughout the experiment. If perfusion pressure at constant flow began to rise, it was assumed that the perfusate had permeated into the vessel and increased resistance to flow. This occurred only in preliminary experiments with 5-week-old rats in which flows higher than 6.0 ml/min/100 g hindquarter weight were attempted. None of the rats reported in this study showed evidence of edema formation.

Results
Blood Pressure and Body Weights
Table 1 summarizes measurements of mean arterial pressures obtained in anesthetized rats just prior to the acute study. There were no differences in mean arterial pressure between the control and prehypertensive 5-week-old animals of either group. The difference between the normotensive and the hypertensive animals was significantly different in the two older groups (p < 0.05), and the difference increased with age in both groups.

Table 2 summarizes the total and hindquarter weight of the hypertensive and normotensive rats at each age

<table>
<thead>
<tr>
<th>Table 1. Arterial Pressure</th>
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<tr>
<td>Age (wks)</td>
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<tr>
<td>5</td>
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<tr>
<td>n = 9</td>
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<tr>
<td>9 &amp; 11</td>
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<td>19 &amp; 17</td>
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<tr>
<td>n = 12</td>
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</tbody>
</table>

Entries are mean systemic arterial pressure in mm Hg. Values are means ± SE.
*Values are significantly different from the respective control (p < 0.05).

<table>
<thead>
<tr>
<th>Table 2. Body and Hindquarter Weight</th>
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<tbody>
<tr>
<td>Body weight</td>
</tr>
<tr>
<td>Spontaneous</td>
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<td>WKY</td>
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<tr>
<td>S</td>
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<td>Age (wks)</td>
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<td>S</td>
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<td>19 &amp; 17</td>
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<tr>
<td>(n = 13)</td>
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<td>(n = 11)</td>
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</table>

Entries are in grams and given as means ± SE.
*Value is significantly different from the respective control (p < 0.05).
studied. At 5 weeks, the normotensive and hypertensive body and hindquarter weights in each group were not significantly different. This was also true at 9 and 11 weeks of age. However, by 19 weeks the body weights of the spontaneously hypertensive rats were significantly less than their respective WKY controls. The SHR hindquarter, on the other hand, was not significantly less in weight than the WKY. There was no significant difference between body or hindquarter weights of S and R rats at 17 weeks.

**Norepinephrine Sensitivity**

The threshold to norepinephrine of both groups at each age studied is shown in table 3. There was no difference between the hypertensive and normotensive threshold response to norepinephrine in either group at any age examined. The baseline values for SHR and WKY respectively prior to norepinephrine administration were: 5 weeks, 5.9 ± 0.5, 4.7 ± 0.9 (p < 0.25); 9 weeks 6.5 ± 0.2,* 4.5 ± 0.3 (*p < 0.001); 19 weeks 6.4 ± 0.2,* 5.1 ± 0.8 (*p < 0.001). Baseline values for Dahl S and R respectively were: 5 weeks, 6.1 ± 0.8, 5.1 ± 0.8 (p < 0.5); 11 weeks 7.3 ± 0.6, 5.6 ± 0.6 (p < 0.1); 9 weeks 9.4 ± 1.1,* 5.5 ± 0.2 (*p < 0.001). The norepinephrine dose-response curve between 0.6 and 10 μg/ml norepinephrine is shown for spontaneous hypertension in figure 1 and for Dahl hypertension in figure 2. The slope of the norepinephrine dose-response curve was significantly elevated in SHR over WKY at 5 and 9 weeks; at 19 weeks, the SHR slope continued to be elevated over WKY, but the difference was not significant (fig. 1). The ED 50 was not decreased in the SHR compared to controls in any of the age groups studied (fig. 1). The slope of the Dahl S norepinephrine dose-response curve was not different from R at any age (fig. 2). Also, the ED 50 of Dahl S was not different from Dahl R at any age (fig. 2). These data suggest that SHR and Dahl S forms of genetic hypertension were not hypersensitive to norepinephrine under the conditions of this study. The elevated slope of the norepinephrine dose-response curve for SHR of all ages suggests that structural changes secondary to an increase in smooth muscle mass were present in these animals and contributed to an elevated slope even though norepinephrine hypersensitivity was not observed.

**Table 3. Norepinephrine Threshold**

<table>
<thead>
<tr>
<th>Age (wks)</th>
<th>Spontaneous (SHR)</th>
<th>WKY</th>
<th>Dahl (S)</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.13 ± 0.02</td>
<td>0.16 ± 0.07</td>
<td>0.57 ± 0.13</td>
<td>0.60 ± 0.10</td>
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<tr>
<td>(n = 9)</td>
<td>(n = 11)</td>
<td>(n = 11)</td>
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<tr>
<td>9 &amp; 11</td>
<td>0.18 ± 0.01</td>
<td>0.19 ± 0.02</td>
<td>0.20 ± 0.08</td>
<td>0.22 ± 0.07</td>
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<tr>
<td>(n = 9)</td>
<td>(n = 8)</td>
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</tr>
<tr>
<td>19 &amp; 17</td>
<td>0.17 ± 0.01</td>
<td>0.24 ± 0.04</td>
<td>0.20 ± 0.03</td>
<td>0.21 ± 0.03</td>
</tr>
<tr>
<td>(n = 12)</td>
<td>(n = 11)</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
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</tbody>
</table>

Entries are mean norepinephrine threshold in μg/ml. Values are mean ± se.
Flow-Pressure Curves at Maximum Dilation

During papaverine infusion, only two flow-pressure measurements were obtained for the 5-week-old animals because of edema formation at high flows. Four flow-pressure measurements were obtained in the two older age groups. The flow-pressure curve at maximal dilation in spontaneously hypertensive rats and their controls is shown in figure 3. The curve of SHR was significantly elevated over WKY at 5 (p < 0.02), 9 (p < 0.001), and 19 weeks (p < 0.001). The flow-pressure curve at maximum dilation in the Dahl salt-sensitive and resistant rats at 5, 11, and 17 weeks is shown in figure 4. At 5 weeks, the Dahl S curve was not significantly elevated above the R. The Dahl S curve was significantly elevated over the R at 11 (p < 0.01) and 17 weeks (p < 0.001). The difference between S and R increased with increasing age (and length of time on a high sodium diet). Thus, the flow-pressure curve of the hypertensive animals was significantly elevated over the normotensive in both genetic groups at each age except in the 5-week-old Dahl rats.

**Figure 2.** Norepinephrine (NE) dose-response curve for Dahl sensitive (S, —) and resistant (R, ---) controls. Values are mean ± SEM for 5-week-old (n = 11 S, 11 R), 11-week-old (n = 8 S, 7 R), and 17-week-old (n = 8 S, 8 R) rats. The ED$_{50}$ was not different between Dahl S and R at any age (Dahl S and R respectively: 5 weeks, 1.57 ± 0.21, 1.60 ± 0.09; 11 weeks, 1.58 ± 0.20, 1.92 ± 0.12; 17 weeks, 1.57 ± 0.08, 1.73 ± 0.15). The slope of the curve was not elevated in Dahl S over R at any age (Dahl S and R respectively: 5 weeks, 17.4 ± 2.4, 14.8 ± 2.4; 11 weeks, 45.8 ± 6.1, 37.3 ± 4.7; 17 weeks 45.7 ± 6.3, 30.5 ± 3.2).

**Figure 3.** Flow-pressure curves for spontaneously hypertensive rats (SHR, —) and Wistar-Kyoto controls (WKY, ---) at maximal dilation. Values are means ± SEM for 5-week-old (n = 11 SHR, 9 WKY), 9-week-old (n = 7 SHR, 8 WKY) and 19-week-old (n = 13 SHR, 11 WKY) rats. The curve of SHR was significantly elevated over WKY at 5 (p < 0.02), 9 (p < 0.001), and 19 weeks (p < 0.001).
Minimal Vascular Resistance With Age

To make comparisons between age groups, minimal vascular resistance was calculated for each age and animal group using the perfusion pressure value at a constant flow of 3 ml/min/100 g hindquarter weight during maximal dilation (thereby normalizing the resistance values). The relationship of minimal vascular resistance correlated with age is shown in figure 5. Minimal vascular resistance (MVR) increased with age in both SHR and WKY in a parallel fashion (p < 0.001) (Fig. 5, left). The SHR MVR was significantly greater than WKY (p < 0.001). The SHR did not increase more rapidly than the WKY (NS). Minimal vascular resistance also increased with age in Dahl S (p < 0.001), but not in R (fig. 5, right). The Dahl S MVR was elevated above the R (p < 0.001). Dahl S increased more rapidly than R (p < 0.01). Thus, MVR was significantly elevated in both SHR and Dahl S over their respective controls with age. These results suggest that the luminal diameter of the resistance vessels at complete relaxation is less in both SHR and Dahl S than in normotensive controls. Since the luminal diameter at complete relaxation is determined by vessel structure, the hypertensive animals have a vessel structure alteration that increases vascular resistance at complete relaxation. In SHR the structural change was present as early as 5 weeks of age and did not progress. In Dahl S the vascular alteration was not present at 5 weeks of age (prior to the initiation of the high salt diet) but was present in early and established hypertension and progressed during these stages.

Maximal Constrictor Response With Age

The maximal perfusion pressure engendered during infusion of supramaximal doses of vasopressin and barium chloride was divided by the constant flow of 3 ml/min/100 g hindquarter weight to calculate the maximal constrictor response in resistance units. The increase in vascular resistance during maximal vasoconstriction is correlated with age in figure 6. When the maximal vasoconstrictor response, rather than change from baseline, was used for identical calculations the results did not differ. The change in resistance from baseline to maximal response increased with age in both SHR and WKY (p < 0.001) (fig. 6, left). The SHR vasoconstrictor response was significantly greater than WKY (p < 0.001). In Dahl S and R, the change in the vasoconstrictor response also increased with age (p < 0.001) (fig. 6, right). However, Dahl S did not increase more rapidly than R nor was Dahl S elevated above R. Thus, the maximal vasoconstrictor response of SHR was elevated over WKY with age, but Dahl S
was not elevated over R. These results suggest that the smooth muscle content of the SHR hindquarter vasculature (responsible for maximal constriction) was significantly greater than that of WKY at all ages and was responsible for the elevation in minimal vascular resistance of SHR over WKY at all ages. The lack of any difference between the maximal constrictor response of Dahl S over Dahl R suggests that a smooth muscle alteration was not a major contributor to the difference in MVR between Dahl S and R in the older age groups.

Discussion

The major conclusion of this study is that structural alterations are present in both male spontaneous and female Dahl salt-sensitive genetic hypertension, but the origin of the two differ. In spontaneous hypertension, an increase in smooth muscle mass contributes importantly to the structural alterations. In Dahl S hypertension, factors other than smooth muscle enhancement appear to contribute to structural alterations. Further, structural alterations precede frank hypertension in spontaneous hypertension but not in Dahl salt-sensitive hypertension (in the absence of a high salt diet).

There are several aspects of the design of this study that require comment. First, a functional assessment of vascular structural alterations was used, that of determining minimal or maximal vascular resistance in the hypertensive or prehypertensive vascular bed compared to the normotensive. This method allows for a functional measurement of small vessel alterations and does not depend upon histologic determinations which are difficult to use for assessment of small vessel structure. In addition, during histological analysis to determine vessel wall/lumen ratio, the fixative used to preserve tissue may predispose to vessel constriction which makes accurate measurement improbable because maximal dilation of the blood vessels is necessary for accurate determinations. Second, an artificial perfusate was used rather than blood to determine minimal vascular resistance because blood contains circulating humoral substances, such as norepinephrine, which may elevate basal vascular tone and make demonstration of structural alterations at maximal dilation less likely. Third, the hindquarter was studied so that the results could be compared with those previously reported. In addition, studies of the hindquarter vascular are technically feasible in the young animal using an artificial perfusate which was an important consideration in this study.

Collis and Vanhoutte have compared vascular reactivity in isolated perfused kidneys from male and female SHR. They found significantly elevated weights, blood pressures, basal perfusion pressures, norepinephrine maximal response, 5-hydroxytryptamine response, barium chloride maximal response, and response to ACh in the male compared to the female SHR. Therefore, different sexes cannot be expected to give similar responses within the same hypertensive genetic group, let alone between genetic groups. However, the purpose of this study was not to compare the two genetic types of hypertension, but to longitudinally analyze each. In addition, in the study of Collis and Vanhoutte, the mean arterial pressures of the male and female rats were significantly different (male 223: female 179 mm Hg) which could have contributed markedly to the differences noted between the two sexes. The mean arterial pressures of the hypertensive rats in this study showed little difference between the different groups of animals. Nevertheless, a possible role for sex differences between male SHR/WKY and female Dahl S and R cannot be totally excluded.

The observation that structural alterations were present as early as five weeks of age in spontaneously hypertensive rats is in agreement with others. In Dahl salt-sensitive hypertension, on the other hand, structural alterations (analyzed by pressure-flow data at maximal dilation) were not observed in five week old Dahl S. Structural alterations became evident in the two oldest Dahl S age groups after initiation of the high salt diet. This finding supports the work of Takeshita and Mark who observed structural vascular changes late in Dahl S hypertension following earlier enhanced neurogenic constrictor tone.

The maximal constrictor capacity of vascular smooth muscle is proportional to the smooth muscle content of the tissues since an increase in passive wall elements could only slightly increase maximal constriction. The capacity of smooth muscle to contract was tested by using vasopressin and BaCl2 to maximally constrict the blood vessels. The observation that spontaneously hypertensive rats demonstrated a significantly elevated constrictor response over controls at all ages suggests that the structural changes found in these animals are due, at least in part, to smooth muscle hypertrophy and/or hyperplasia. On the other hand,
the failure of the resistance vessels of the Dahl salt-sensitive hindquarters to be higher than that of controls during maximal constriction indicated that the structural alteration in Dahl S was not due to hypertrophy or hyperplasia of the smooth muscle. Rather, a thickening of vascular noncontractile elements such as vessel "waterlogging," an increase in collagen content or intimal thickening apparently contributes to the structural alteration.

In SHR, the increase in smooth muscle mass could be due to an increase in the number of vessels (with an associated increase in smooth muscle/hindquarter weight) or due to an increase in smooth muscle in existing vessels. Evidence from other studies suggests that SHR have fewer vessels rather than a greater number of vessels compared to WKY. In addition, histologic studies indicate that an increased smooth muscle mass in comparison to the vessel lumen is found in SHR. Thus, the enhanced smooth muscle mass in SHR found at all ages can be ascribed to smooth muscle hypertrophy/hyperplasia in existing vessels rather than due to an increased vascularity. The observation that the difference in the minimal vascular resistance between SHR and WKY (fig. 5) does not change with age suggests that the alteration is present early, fixed and not related to an elevation in arterial pressure with age.

The differences noted between the two different types of hypertension at maximal dilation could be related to the high salt diet necessary to induce hypertension in Dahl S. Recent evidence suggests that the noncontractile vascular changes found in Dahl S may also be found in other forms of hypertension on a high salt diet. Limas et al. reported that high salt intake caused marked intimal proliferation in intrarenal arteries in SHR. In humans, Takeshita et al. suggested that high salt intake increased minimal vascular resistance in the forearm of salt-responsive hypertensive individuals. These changes were considered to be caused by vascular "waterlogging." Thus, the response to the high salt diet in Dahl S may not be unique and the differences demonstrated in this study at maximal dilation may be due, in part, to the high salt diet of Dahl S.

In this study, we have used established criteria to determine enhanced sensitivity to norepinephrine, a shift to the left of the dose-response curve indicated by a decrease threshold and/or $ED_{50}$. A change in slope of the dose-response curve is a less regular feature of norepinephrine hypersensitivity and an increased slope in the absence of norepinephrine hypersensitivity is indicative of a structural alteration. In this study, the threshold and $ED_{50}$ were not decreased in SHR or Dahl S compared to controls in any age group. These data suggest that norepinephrine hypersensitivity was not present in these prehypertensive animals compared to controls and correlates with the finding of others in SHR and Dahl S. In SHR, norepinephrine hypersensitivity has been reported when tested in the presence of cocaine, which blocks neuronal uptake of norepinephrine. It is possible that addition of cocaine to the perfusate in this study may have unmasked norepinephrine hypersensitivity in SHR.

Our finding that the slope of the norepinephrine dose-response curve was elevated in SHR in the absence of enhanced sensitivity to norepinephrine, is consistent with the presence of structural alterations in SHR that are secondary to smooth muscle enhancement. In addition, the finding that the slope of the norepinephrine dose-response curve was not elevated in Dahl S in the absence of norepinephrine hypersensitivity strengthens our observation that structural alterations found in Dahl S at maximal dilation are not due to smooth muscle changes.

In summary, vascular structural alterations are present in both male SHR and female Dahl S rats during the development of hypertension. In Dahl S, the structural alteration does not develop until after the initiation of the high salt diet but then it progresses with age and length of time on the diet. In SHR, the structural alteration is present as young as five weeks of age and progresses with age paralleling WKY so the difference between SHR and WKY does not change with age. Smooth muscle hypertrophy/hyperplasia appears to contribute to the vascular structural alteration in SHR, but not in Dahl S. Thus, structural alterations with different developmental characteristics and origins contribute to the elevated vascular resistance found in different types of genetic hypertension.

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HINDQUARTER VASCULATURE DURING DEVELOPING GENETIC HYPERTENSION


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Longitudinal study of the hindquarter vasculature during development in spontaneously hypertensive and Dahl salt-sensitive rats.

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