Effects of Hypertension and Aging on Coronary Arteriolar Density

John C. Vitullo, Marc S. Penn, Karel Rakusan, and Pierre Wicker

Coronary reserve has been shown repeatedly to be depressed in hypertension and aging. The underlying mechanisms remain elusive, but structural alterations of the coronary vasculature have been implicated. In this study, we measured maximal coronary dilator capacity and structural characteristics relevant to coronary resistance in aging normotensive (Wistar-Kyoto, n=22) and spontaneously hypertensive rat (SHR) strains (n=25) at 1.5, 4, 11, 16, and 22 months of age. Coronary flow measurements, using radiolabeled microspheres, demonstrated a significant (p<0.01) hypertension- and age-related decline in maximal coronary dilator capacity. After flow measurements, vascular dimensions and arteriolar density were obtained from 1-μm sections prepared from perfusion-fixed hearts. A total of 10,012 arterioles were analyzed, 4,820 in hypertensive and 5,192 in normotensive rats. There was an 18-28% reduction in arteriolar density in hypertensive rats that specifically affected the terminal arteriolar bed at 1.5-11 months. However, the decrement in arteriolar density stabilized at 10% and 6% in 16- and 22-month-old hypertensive rats, respectively. Arteriolar density was not affected by aging. In both strains, there was a significant (p<0.01) age-related decrease in the ratio of lumen diameter to wall thickness in arterioles >50 μm. In addition, there was an overall 30% decrease (p<0.01) in the ratio of lumen diameter to wall thickness in hypertensive compared with normotensive rats. These data indicate that both hypertension and aging are accompanied by structural alterations of the coronary resistance vasculature. These structural alterations may contribute to the depression in coronary reserve that complicates hypertension and aging. (Hypertension 1993;21:406–414)

Key Words: aging • hypertension, essential • arterioles • vascular resistance

There is increasing evidence that the cardiovascular consequences of aging and hypertension are similar in many respects,1,2 an observation that may account for the greater severity of hypertension in the elderly population.3 As a result, a precise delineation of the biological changes that occur during the aging and hypertensive processes or an improved understanding of the mechanisms underlying these alterations may have important clinical implications and offer new therapeutic perspectives. Various studies have reported that coronary reserve, which reflects the ability of the coronary circulation to dilate in response to a stimulus, was depressed in aging and hypertension.4-7 The mechanisms responsible for this limitation of myocardial perfusion remain elusive, although alterations in the functional or structural characteristics of the coronary resistance vessels have been postulated. Smooth muscle cell abnormalities or an altered endothelial modulation of vascular tone have been described during hypertension and aging, resulting in a diminished response to vasodilators.2,4 Several studies have also reported the presence of structural alterations, including vascular hypertrophy, luminal narrowing, or both, in hypertensive hearts both in humans9,10 and in animals.11,12 However, there is a dearth of precise morphometric studies of the coronary vasculature, and the suggestion that alterations in coronary vascular geometry represent a characteristic feature of hypertensive cardiac disease has indeed been challenged in a recent report.13 The possibility of vascular rarefaction as an alternate mechanism for the reduced coronary reserve has been raised5-14 but has never been tested rigorously in either hypertension or aging because of the relative paucity of arterioles on histological cross-sections. Extensive sampling of the myocardium is required, and as a result very few laboratories have undertaken this task. In addition, many studies have been restricted to short-term hypertension. Because human hypertension typically spans one or several decades, information regarding the onset and development of coronary structural alterations from the initial to the late phase of the process would be of particular clinical relevance.

The aim of the present investigation was to examine the hypothesis that alterations in arteriolar density and thickness participate in the development of coronary reserve abnormalities associated with long-term hypertension and aging. To this end, maximal coronary dilator capacity, wall thickness, lumen diameter, and arterial density were simultaneously measured in spontaneously hypertensive rats (SHR) and their normoten-
sive Wistar-Kyoto (WKY) controls at various stages from early adulthood to senescence. SHR were selected because of their relatively prolonged life expectancy and the long duration of hypertension that resembles the course of human hypertension. Furthermore, these biological characteristics are unique in that the time course of alterations in the coronary vasculature and the influence of hypertension or aging on these changes can be readily determined at numerous time points throughout the life span of the animal. Because resistance is inversely proportional to the fourth power of lumen radius, we surveyed a large number of arterioles to improve sampling precision and detect small but physiologically relevant changes in lumen diameter or the ratio of lumen diameter to wall thickness.

Methods

Age-matched male SHR and WKY rats were obtained from Taconic Farms, Germantown, N.Y., maintained in our facility (approved by the American Association for the Accreditation of Laboratory Animal Care), and sampled at 1.5, 4, 11, 16, and 22 months of age. All rats were housed three to a cage and fed normal Purina chow pellets (0.4% sodium content) ad libitum.

Coronary Flow Measurements

Coronary flow was determined in conscious, unrestrained rats using left atrial injections of radioactive microspheres. Briefly, the rats were initially instrumented with a left atrial catheter. After a 3–4-day recovery period and 3–5 hours before the flow measurements, another catheter (PE-50) was inserted with rats under ether anesthesia into the right femoral artery and injected into the left atrium over a 15-20-second period. Starting 10 seconds before the injection, blood withdrawal rate (0.26 mL/min) was used to minimize the ratio between the mean arterial pressure measured and the coronary blood flow to return to baseline levels (478 ± 27 mL/min per 100 g, n = 5, 3 hours after 18 mg/kg carbochrome versus 448 ± 58 mL/min per 100 g under baseline conditions in another group of normotensive rats, n = 8, p = NS).

On completion of the flow measurements, the rats were anesthetized with pentobarbital (50 mg/kg) administered via the left atrial catheter. The animals were then intubated and ventilated with room air using a Harvard small-animal respirator. The abdominal aorta was exposed through a midline incision and cannulated with a 22G catheter. The heart were perfused in situ with 200–250 mL phosphate-buffered saline solution adjusted to pH 7.4 and containing 2% procaine to produce maximal coronary dilatation as previously described,15,16,17 After cardiac arrest in diastole, both SHR and WKY hearts were fixed at a constant pressure of 100 mm Hg with approximately 150 mL of 1.5% glutaraldehyde buffered to pH 7.4 with phosphate buffer. The chest was opened and the heart removed for further dissection. After the proper location of the left atrial catheter was confirmed, the atria were discarded and the right ventricular free wall was separated from the left ventricle. The left ventricle was bisected perpendicularly to the long axis at one third the distance between the base and apex. A 2-mm-thick slice of the entire cross-sectional surface of the left ventricle was obtained and diced into approximately 10 smaller samples for histological processing. The remainder of the left ventricle was divided into septum and the subendocardial and subepicardial halves of the free wall. All tissue samples were weighed, suspended in the fixative solution, and counted overnight along with the reference sample in a gamma counter (model 5010, Packard Instrument Co., Inc., Downers Grove, Ill.). Thus, the left ventricular coronary blood flow values reported in this article include flows to the samples used for the histological studies. Coronary blood flow was calculated with a computer program correcting for background, decay, and spillover between the energy windows of each isotope.15 Coronary conductance was computed as the ratio between the mean arterial pressure measured just before each microsphere injection and the coronary blood flow.

Morphometric Studies

The left ventricular tissue samples selected for the histological studies were washed for 1 hour in two changes of 0.13 M phosphate buffer (pH 7.35) containing 7.5% sucrose and were post-fixed for 2 hours at 4°C in 1% OsO₄ in the same buffer. The samples were then dehydrated from 50% ethanol and embedded in Spurr's low-viscosity resin with all specimens oriented identically. For light microscopy, 1-μm-thick sections were cut (LKB-III ultramicrotome, LKB, Rockville, Md.). Sections were mounted on glass slides and stained with 1% toluidine blue in 1% borax.

All sections from one rat were given an identification number unrelated to the strain and age of the animal and were read in a blind fashion by a trained observer.
(J.V.). Sections were projected onto a monitor with an Olympus BH-2 light microscope connected to a video camera yielding a final magnification of ×3,000. For first- and second-order arterioles, final magnification was ×1,200.

Arterioles were identified based on the following criteria: 1) at least one complete layer of vascular smooth muscle cells, 2) round or oval profile indicative of a fairly rigid wall, 3) presence of elastic fibers, and 4) wall thickness larger than that of a venule of comparable size. Once identified as an arteriole, three measurements were determined on a digitizing pad with BIOQUANT IV morphometry software (R&M Biometrics, Nashville, Tenn.): 1) external major: the adventitia-to-adventitia distance of the long axis of the vessel, 2) external minor: the adventitia-to-adventitia distance of the short axis of the vessel, and 3) internal minor: the endothelium-to-endothelium distance of the short axis of the vessel. Approximately 150–250 arterioles per heart were analyzed. From these basic histometric data, wall thickness, lumen diameter, and the ratio of lumen diameter to wall thickness were computed. Wall thickness was defined as one half of the external minor minus the internal minor diameter.

### Statistical Analysis

Values are mean±SD. A two-way analysis of variance (ANOVA) using a general linear model was performed to statistically assess the effects of strain, age, and their interaction. When overall effects were detected by the two-way ANOVA, post hoc pairwise comparisons were performed using linear comparisons of least-squares means. The following pairwise comparisons were made: 1) between both strains within each age group and 2) between 1.5-month-old animals and older age groups within each strain. The level of significance was corrected for the number of comparisons according to the Bonferroni inequality.

To assess whether structural alterations are confined to a specific segment of the coronary vasculature or represent a generalized defect, we grouped the arterioles into five size classes based on their external minor diameters (<20, 20.1–30, 30.1–40, 40.1–50, and >50 μm). The size class was introduced in the statistical model as a covariate, and the effects of aging and hypertension on vascular geometry and arteriolar density were again evaluated. This analysis revealed a highly significant (p<0.001) interaction between aging or hypertension on the one hand and lumen diameter on the other hand, indicating that the effects of age and hypertension are significantly different for arterioles of different diameters.

### Table 1. Body Weight, Blood Pressure, and Left Ventricular Mass

<table>
<thead>
<tr>
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<th>Age (months)</th>
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<tr>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td>WKY</td>
<td>n=5</td>
</tr>
<tr>
<td>SHR</td>
<td>n=5</td>
</tr>
<tr>
<td>Body weight (g)†‡∥†‡∥<em>†‡∥</em>†‡∥</td>
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<tr>
<td>WKY</td>
<td>133±20</td>
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<tr>
<td>SHR</td>
<td>102±8</td>
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<tr>
<td>Mean arterial pressure (mm Hg)§</td>
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<tr>
<td>Baseline†‡∥*†‡∥</td>
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<tr>
<td>WKY</td>
<td>98±10</td>
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<tr>
<td>SHR</td>
<td>131±15</td>
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<tr>
<td>Carbochrome low dose*†‡∥</td>
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<tr>
<td>WKY</td>
<td>96±8</td>
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<tr>
<td>SHR</td>
<td>117±16</td>
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<td>WKY</td>
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<td>SHR</td>
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<tr>
<td>Left ventricle</td>
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<tr>
<td>Weight (mg)†∥</td>
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<tr>
<td>WKY</td>
<td>317±57</td>
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<tr>
<td>SHR</td>
<td>292±16</td>
</tr>
<tr>
<td>Weight/body weight (mg/g)†‡∥*†‡∥</td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>2.38±0.16</td>
</tr>
<tr>
<td>SHR</td>
<td>2.85±0.15</td>
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</table>

WKY, Wistar-Kyoto rat; SHR, spontaneously hypertensive rat. Carbochrome low dose and high dose: 0.45 mg/kg per minute up to 12 mg/kg and 0.675 mg/kg per minute up to 18 mg/kg, respectively. Values are mean±SD.

* p<0.01, effect of strain by two-way analysis of variance.
† p<0.01, effect of aging by two-way analysis of variance.
∥ p<0.01, interaction between effects of strain and aging by two-way analysis of variance.
§ Changes in mean arterial pressure with carbochrome were highly significant (p<0.001) by two-way analysis of variance with repeated measures.
| p<0.05, effect of aging by two-way analysis of variance.
### Table 2. Coronary Hemodynamics at Maximal Vasodilatation

<table>
<thead>
<tr>
<th></th>
<th>Age (months)</th>
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<th>11</th>
<th>16</th>
<th>22</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Left ventricular coronary blood flow (mL/min per 100 g)</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Carbochrome low dose†</td>
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<td></td>
</tr>
<tr>
<td>WKY</td>
<td>1,911±434</td>
<td>1,475±223</td>
<td>1,216±237</td>
<td>837±212</td>
<td>875±182</td>
</tr>
<tr>
<td>SHR</td>
<td>1,617±476</td>
<td>1,305±160</td>
<td>913±124</td>
<td>790±156</td>
<td>733±54</td>
</tr>
<tr>
<td>Carbochrome high dose†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>1,819±434</td>
<td>1,182±281</td>
<td>1,339±152</td>
<td>1,078±163</td>
<td>588±48</td>
</tr>
<tr>
<td>SHR</td>
<td>1,866±283</td>
<td>1,364±387</td>
<td>896±79</td>
<td>833±215</td>
<td>713±167</td>
</tr>
<tr>
<td><strong>Conductance (mL/min per 100 g per mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbochrome low dose†</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>WKY</td>
<td>19.8±3.8</td>
<td>17.8±4.2</td>
<td>14.2±3.9</td>
<td>13.4±2.8</td>
<td>10.1±2.7</td>
</tr>
<tr>
<td>SHR</td>
<td>13.9±3.9</td>
<td>10.2±0.9</td>
<td>7.2±0.9</td>
<td>8.3±1.4</td>
<td>7.1±0.4</td>
</tr>
<tr>
<td>Carbochrome high dose†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>20.4±6.1</td>
<td>15.0±4.9</td>
<td>16.2±0.7</td>
<td>11.5±4.0</td>
<td>7.5±1.2</td>
</tr>
<tr>
<td>SHR</td>
<td>16.6±1.7</td>
<td>11.1±2.7</td>
<td>7.8±0.9</td>
<td>9.1±2.0</td>
<td>6.8±0.7</td>
</tr>
</tbody>
</table>

WKY, Wistar-Kyoto rat; SHR, spontaneously hypertensive rat. Carbochrome low dose and high dose: 0.45 mg/kg per minute up to 12 mg/kg and 0.675 mg/kg per minute up to 18 mg/kg, respectively. Values are mean±SD. No significant differences were found between the two doses of carbochrome by two-way analysis of variance with repeated measures.

*^p<0.01, effect of strain by two-way analysis of variance.
†p<0.01, effect of aging by two-way analysis of variance.

Blood pressure on coronary structural properties were not identical for all vessel sizes. Therefore, the effects of age and blood pressure were subsequently examined in each size class with an ANOVA, as described above.

Differences between precarbochrome and postcarbochrome values were statistically assessed using an ANOVA with repeated measures.

All statistical calculations were performed on a microcomputer using the SAS statistical package.

### Results

**Body Weight, Left Ventricular Mass, and Blood Pressure**

As expected, intra-arterial mean blood pressure was significantly (*p<0.001*) elevated in SHR compared with WKY rats (Table 1). Left ventricular weight increased significantly (*p<0.001*) during aging, but no differences between strains were noted. However, because body growth in SHR was less than in WKY rats, left ventricular mass corrected for body weight, an index of cardiac hypertrophy, was significantly larger in the hypertensive strain (*p<0.01*).

**Maximal Coronary Flow Measurements**

Changes in maximal coronary flow and conductance were identical after the low and high dose of carbochrome (Table 2). Both parameters decreased markedly during the aging process in both SHR and WKY rats. When differences between strains were examined, maximal coronary conductance was found to be significantly depressed in SHR compared with WKY rats.

**Morphometric Data**

Before quantitative analysis was performed, all tissue sections were examined to ensure that vessels had been fixed in the relaxed state, as judged by smooth luminal outlines and internal elastic laminae. The total number of vessels surveyed was 10,012: 4,820 in SHR and 5,192 in WKY rats.

**Arteriolar Density**

There was an overall significant (*p<0.01*) decrement in arteriolar density in hypertensive rats by an ANOVA, whereas the effects of aging were of borderline significance (*p=0.055*, Figure 1). The differences associated with hypertension were noted in all age groups but tended to diminish with aging. At 1.5 months, arteriolar density in WKY rats was 3.95 compared with 2.80

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**FIGURE 1.** Bar graph shows myocardial arteriolar density in Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR). Data are presented as mean±SEM for each strain and age. There was an overall statistically significant (*p<0.01* by analysis of variance) difference between SHR and WKY. Arteriolar density in SHR tended to return to control values in older animals, but this trend was not significant.
arterioles per square millimeter in SHR, a 28% decrease. At 4 months and 11 months, arteriolar density was reduced by 18% and 25%, respectively; it was decreased only by 10% and 6% in 16- and 22-month-old SHR, respectively. However, this trend toward smaller differences in senescent rats was not significant.

A separate analysis of arteriolar density in each size class revealed that arteriolar density was significantly (p<0.01) reduced by 25% in SHR in arterioles smaller than 20 μm in diameter (Figure 2). Other size classes did not exhibit any significant changes (Figure 2). No consistent age-related alterations in arteriolar density were observed in each separate size class.

**Wall Thickness and Lumen Diameter**

Figure 3 illustrates the internal lumen diameter, mean wall thickness, and ratio of lumen diameter to wall thickness of coronary arterioles grouped according to age and strain with all vessel sizes included. Although an ANOVA revealed an overall significant (p<0.05) effect of aging on lumen diameter (Figure 3, top panel) and lumen diameter-to-wall thickness ratio (Figure 3, bottom panel), the changes were not consistent across age groups, and no general trend toward an increase or decrease in vascular dimensions could be demonstrated. The only exception was the lumen diameter in SHR, which was significantly (p<0.05) higher in the 1.5-month-old rats compared with all other groups (Figure 3, top panel). Wall thickness was 12–28% greater in SHR compared with WKY rats (p<0.025), with the exception of 1.5-month-old animals, in whom values were nearly identical (Figure 3, middle panel). These differences, however, were no longer apparent when wall thickness and lumen diameter were expressed as a ratio (Figure 3, bottom panel), because vascular diameter was moderately but significantly (p<0.01) larger in SHR (Figure 3, top panel); however, this vascular enlargement was mostly seen in the younger hypertensive animals (Figure 3, top panel).

A separate analysis in each size class showed that hypertension-related wall thickening was limited to arterioles >50 μm (Figure 4). Wall thickness was 22% greater (p<0.025) in SHR than in WKY rats, whereas the lumen diameter-to-wall thickness ratio was decreased by 30% (p<0.01; Figure 4, middle and bottom panels). The larger arterioles also exhibited a significant (p<0.01) age-related increase in wall thickness, which was particularly evident when wall thickness was normalized for the lumen diameter (Figure 4, bottom panel). There were no consistent age-related changes in vascular geometry in the other size classes.
Aging and Coronary Arteriolar Density

1.3 3 11 16 22
AGB (month*)

1 2 3 4 5
AGE (months)

FIGURE 4. Bar graphs show mean lumen diameter (top panel), wall thickness (middle panel), and lumen-to-wall thickness ratio (bottom panel) of coronary arterioles >50 μm in diameter from Wistar-Kyoto (WKY) rats (open bars) and spontaneously hypertensive rats (SHR) (hatched bars). There was a significant \( p<0.01 \) by analysis of variance) increase in wall thickness whether the latter was expressed in absolute terms (middle panel) or normalized for lumen diameter (bottom panel) in SHR. All three parameters changed significantly \( p<0.05 \) by analysis of variance) with aging. \* \( p<0.05 \) vs. strain-matched 1.5-month-old animals by linear comparisons of least-squares means.

Arteriolar Frequency Distribution

Figure 5 shows the frequency distribution of arterioles according to their lumen diameter class. Sixty-four percent of all arterioles in WKY rats and 58% of all arterioles in SHR were found in the smaller diameter class (<20 μm). These differences in arteriolar distribution were significant at the level of \( p<0.01 \) by \( \chi^2 \) analysis.

Discussion

This morphometric investigation demonstrates that both aging and mid- to long-term hypertension are associated with a remodeling of the coronary resistance vessels that is specifically localized to the larger arterioles. This study also provides evidence for an early microvascular rarefaction (on a unit mass basis) involving the coronary terminal arterioles of hypertensive hearts. This reduction in arteriolar density was present at an early stage in the development of hypertensive heart disease but tended to become less prominent after long-term hypertension and hypertrophy.

One of our main objectives was to assess whether a structural redesign of the coronary resistance network could account for the coronary reserve abnormalities characteristic of hypertension and aging. Therefore, the approach selected in this investigation combined flow measurements with a simultaneous evaluation of structural parameters relevant to coronary resistance (see discussion below).

Arteriolar Density: Effects of Hypertension and Aging

The decrement in arteriolar density noted in the present study in genetically hypertensive animals is consistent with another investigation of renovascular hypertensive rats by our group, although in that study the differences did not reach statistical significance. Similarly, Breisch et al reported a 34% reduction in arteriolar density in pigs with cardiac hypertrophy secondary to aortic banding. In their study, only arterioles ranging from 25 to 75 μm in diameter were included. In contrast, Tomanek et al could not document any significant differences in arteriolar density between dogs with 7-month one-kidney, one clip renovascular hypertension and their normotensive controls. Reasons for these divergent conclusions between the work of Tomanek et al and other studies, including the present one, are unclear but may be related to species differences as well as to methodological factors such as the experimental model and duration of hypertension. An important methodological feature of our study was the...
very large number of vessels analyzed, thereby minimizing the risk of a beta error.

In WKY rats, arteriolar density remained constant during maturation and senescence. Because in rats myocyte size continuously expands until a relatively advanced age,24-26 this finding suggests that arteriolar growth accompanies cardiac growth and aging in an eminently coordinated fashion, resulting in the maintenance of a stable relation between vessel number and myocardial mass throughout the life span of the animal. However, in SHR this vascular adaptation to myocyte growth may be compromised. Cardiac mass and myocyte size have been reported to increase twofold to threefold from early to mid-adulthood in this genetic model of hypertension.13,17,24-26 In our study, arteriolar density was reduced but only moderately (18-28%) in SHR compared with WKY rats. These combined observations suggest that, although some coronary arteriolar growth occurs during the course of hypertension, the pace of this adaptive process is of an insufficient magnitude to parallel myocyte enlargement and maintain arteriolar vascularity. This abnormality appears to be a characteristic feature of pathological hypertrophy induced by pressure overload.2,5

Our observation of a vascular rarefaction (on a unit mass basis) predominantly localized to the precapillary resistance vasculature is not unique to the coronary circulation and has been previously reported in arterioles from SHR skeletal muscle.12,27 These findings have been ascribed to prolonged sympathetic drive maintaining the vessels in a closed position and eventually resulting in their atrophy.27 However, no definite proof of this mechanism has been provided.

Vascular Remodeling in Hypertension and Aging

Several studies have quantitatively analyzed vascular geometry in coronary resistance vessels from animals subjected to various experimental forms of hypertension. Two investigations in dogs with renovascular hypertension could not demonstrate any significant changes in the coronary lumen diameter-to-wall thickness ratio.13,17 However, the majority of studies have demonstrated that coronary arterioles undergo hypertrophy in genetic,28 acquired renovascular,16,22 and deoxycorticosterone acetate-salt29 hypertension. The present investigation confirms these results and shows that this vascular remodeling is predominantly localized to the larger segments of the coronary vasculature in SHR. However, this finding may be model specific, because in other experimental types of hypertension vascular hypertrophy has been reported to occur in smaller segments.13,17,22,29

It has been postulated that the vascular remodeling associated with hypertension constitutes an adaptive response to long-term elevations of intraluminal pressure that eventually result in a normalized wall stress.19,20 This hypothesis is supported by observations from hemodynamic and morphometric studies in peripheral beds showing that both the blood pressure elevation characteristic of hypertension50 and medial hypertrophy51-53 are predominantly localized to the larger arterioles. The distribution of resistance across the coronary vasculature has not been precisely measured because of technical limitations. However, studies in normotensive animals suggest that nearly 50% of coronary resistance resides in the large and middle-sized arterioles.34,35 Whether a similar resistance pattern is present in hypertensive animals remains to be determined, but the finding of a decreased ratio of lumen diameter to wall thickness involving predominantly the arterioles >50 µm in SHR (Reference 34 and the present study) is consistent with the conclusion that the increase in systemic blood pressure may be transmitted only to this segment of the coronary circulation. It is well acknowledged, however, that numerous factors determine the functional and structural response of the peripheral vasculature to hypertension.2,5,19,20 Mechanisms other than an increased load may modulate vascular remodeling, depending on the model and duration of hypertension or the age at which the pressure overload is imposed, as suggested by a recent study.16 The factors involved in these microvascular alterations remain elusive (for review, see Reference 2).

Role of Structural Alterations in the Diminished Coronary Reserve of Hypertension and Aging

Our flow measurements are in full agreement with earlier experimental investigations showing an age-related4,6,8,36 or hypertension-related4,7,13,14,37-39 decline in coronary reserve. Whether these functional abnormalities can be ascribed in full or in part to arteriolar rarefaction and vascular remodeling cannot be definitively determined from our study. However, theoretical and experimental work by Folkow's group4,21 may help shed some light on this issue. Their studies indicate that decreases in the ratio of lumen diameter to wall thickness are accompanied by an elevation in minimal resistance of a similar magnitude. On a theoretical basis, a reduction in the total coronary cross-sectional area secondary to a decrement in the number of vascular channels (rather than a decrease in each channel cross-sectional area) may similarly result in a diminished coronary reserve. Hallback et al21 have experimentally validated this prediction by showing that obstruction of approximately 55% of precapillary arterioles with 50-µm microspheres results in a 54% reduction in the functionally significant vascular bed. These observations suggest that the combined abnormalities in arteriolar density and wall thickness found in our study may account for a substantial fraction of the deficit in coronary reserve associated with aging and hypertension. Interestingly, it is conceivable that these structural changes exerts additive effects on coronary resistance, because they involved different segments of the coronary vascular tree.

The respective participation of vascular remodeling and rarefaction may be modulated again by the nature and duration of the inciting stimulus. In young hypertensive animals, vascular rarefaction may be predominantly responsible for the reduction in coronary reserve, because the ratio of the lumen diameter to wall thickness was normal (Figure 4, bottom panel) whereas arteriolar density was clearly reduced (Figure 1). After a prolonged period of hypertension and cardiac hypertrophy, some arteriolar neoformation may occur, which could then attenuate the decline in coronary reserve due to vascular rarefaction. However, during the same period vascular remodeling with a relative wall thickening occurs, possibly as a consequence of the long-term augmentation in intravascular load or a more direct
effect of aging. This vascular redesign may then nullify the beneficial consequences of vascular growth and possibly result in further deterioration of coronary reserve.

A decrease in the ratio of lumen diameter to wall thickness has been previously reported in aging human and rat aorta. The apparent parallelism between age-related changes in blood pressure and the ratio of lumen diameter to wall thickness in the present study in both WKY rats and SHR with aging (Figure 4, bottom panel, and Table 1) suggests that this structural alteration may also be due to repeated hemodynamic stresses on the vascular wall throughout the animal's life. In contrast to hypertension, vascular remodeling appears to represent the most significant structural mechanism involved in the age-related depression in coronary reserve. There were no consistent changes in arteriolar density in aging animals, a finding that was particularly apparent in WKY rats (Figure 1). These structural differences between hypertension and aging may reflect the greater role of functional factors in the depression of coronary reserve due to aging.

**Advantages and Limitations of the Present Study**

Our study has several advantages and limitations that require additional discussion.

Maximal coronary flow was measured in each rat after the administration of carbochrome using two different infusion protocols. This experimental approach was selected to ensure that the coronary bed had been maximally dilated before flow measurements and that the conclusions of the study were not affected by the dose and modalities of administration of carbochrome. The lack of differences between the two doses of carbochrome demonstrates that maximal vasodilation was effectively reached in all instances. Furthermore, comparison of the flow values with previous work from other laboratories indicates that carbochrome is a more potent vasodilator than dipyridamole, another vasodilator commonly used in rat studies.

Another advantage of the present study over earlier investigations is that a very large number of arterioles were surveyed. This large sample size ensured that wall thickness and lumen diameter values were estimated in each animal with a precision better than 3% at the 95% confidence interval. Additionally, extensive sampling enabled us to perform a complete histometric analysis of the entire coronary hierarchy from the largest to the terminal arterioles. This was felt an important consideration because arteriolar responses to a disease combining mechanical and neurohumoral alterations such as hypertension may be heterogeneous and modulated by the anatomic location within the vascular network.

One potential limitation is the use of a similar fixation pressure in SHR and WKY rats unlike the in vivo situation. However, because our intent was to measure the ratio of the lumen diameter to wall thickness, a structural characteristic relevant to vascular resistance, it was important to minimize hemodynamic differences at the time of fixation to allow meaningful interpretations of the anatomic data. This approach has been previously selected by other investigators. Furthermore, it should be noted that the use of a fixation pressure close to in vivo levels in SHR would not have affected the major conclusion of our study regarding vascular remodeling. As a result of the higher fixation pressure, the ratio of lumen diameter to wall thickness would have increased in SHR, and differences between SHR and WKY rats would have been blunted. However, even under these circumstances vascular hypertrophy would have been the only possible interpretation of a seemingly unchanged wall thickness in the face of a larger distending pressure.

We selected short-axis dimensions to estimate lumen diameter. This measurement minimizes the effects of section obliquity but assumes a circular shape. Variations in vascular profile may therefore have introduced spurious differences between experimental groups. However, the lack of significant differences in the ratio of external major to external minor dimensions between strain or age groups (data not shown) suggests that this is an unlikely possibility.

We did not measure arteriolar length, which is another important parameter relevant to vascular resistance. Arteriolar length cannot be obtained directly. Some attempts have been made at indirectly estimating this parameter based on geometrical models of the coronary circulation. The accuracy of these models, however, relies on the assumption that coronary arterioles run a straight course in the myocardium, an assumption that is not always verified.

The validity of the WKY rat as an appropriate control has been questioned recently. To gain further insight on this issue, we compared coronary reserve in 3-month-old WKY (Taconic Farms), Sprague-Dawley, and normotensive Wistar (Hilltop Lab Animals, Inc., Scottsdale, Pa.) male rats. No significant differences in coronary reserve were found among the three strains (maximal conductance, 17.5±2.2, 17.2±3.0, and 15.7±3.5 mL/min per 100 g per millimeter of mercury, respectively), demonstrating that the WKY is an appropriate normotensive control for coronary circulation studies.

In summary, these data indicate that both hypertension and aging are accompanied by structural alterations of the coronary resistance vasculature. This vascular redesign appears to be modulated by both the nature and duration of the inciting stimulus, as well as vessel position in the coronary hierarchy, and may contribute to the depression in coronary reserve that accompanies hypertension and aging.

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