Impaired Diastolic Function and Coronary Reserve in Genetic Hypertension
Role of Interstitial Fibrosis and Medial Thickening of Intramyocardial Coronary Arteries

Christian G. Brilla, Joseph S. Janicki, and Karl T. Weber

Left ventricular hypertrophy (LVH) in rats with genetic hypertension is accompanied by abnormal myocardial diastolic stiffness and impaired coronary reserve. Whether these functional defects are related to a structural remodeling of the myocardium that includes an interstitial and perivascular fibrosis, myocyte hypertrophy, and medial thickening of intramyocardial coronary arteries is uncertain. To address these issues, 14-week-old male spontaneously hypertensive rats with established hypertension and LVH were treated with low-dose (SLO group: 2.5 mg/kg/day, n=11) or high-dose (SHI group: 20 mg/kg/day, n=9) oral lisinopril for 12 weeks to sustain hypertension and LVH or to normalize arterial pressure and myocardial mass, respectively. When SHI and SLO groups were compared with age- and sex-matched 26-week-old untreated spontaneously hypertensive rats (n=11) and normotensive Wistar-Kyoto rats (n=9), we found 1) normalization of blood pressure (p<0.005) and complete regression of LVH (p<0.005) in the SHI group and no significant blood pressure or LVH reduction in the SLO group, 2) complete regression of morphometrically determined myocardial interstitial and perivascular fibrosis in SHI and SLO groups (p<0.025) associated with normalization of diastolic stiffness, measured in the isolated heart (p<0.025), and 3) regression of medial wall thickening of intramyocardial coronary arteries only in the SHI group (p<0.005), accompanied by a normalization of coronary vasodilator reserve to adenosine (p<0.005). Thus, interstitial fibrosis and not LVH is responsible for abnormal myocardial diastolic stiffness, whereas medial wall thickening of intramyocardial resistance vessels, influenced by arterial pressure, is associated with impaired coronary reserve. (Circulation Research 1991;69:107–115)

In the rat with renovascular or genetic hypertension, previous studies have demonstrated that in addition to left ventricular hypertrophy (LVH), mediated by an increment in cardiac myocyte size, there may also be an accumulation of fibrillar collagen within the interstitium and the adventitia of intramyocardial coronary arteries together with a medial thickening of these vessels. This structural remodeling of the extracellular space has been held responsible for abnormalities in myocardial stiffness and the vasomotor reactivity of intramyocardial resistance vessels. Alternatively, it has been argued that the hypertrophic growth of cardiac myocytes alone accounts for these functional abnormalities. Previous studies have sought to address these issues through an examination of the hypertrophic process, including its development, prevention, or regression. Because it has not been possible to dissociate pertinent variables in these studies, the relative contribution of the myocyte and nonmyocyte compartments to the appearance of pathological LVH with abnormal myocardial stiffness and coronary reserve remains unclear. The in vitro myocyte preparation has been used to gain a better understanding of this issue. However, the simple elegance of the isolated myocyte eliminates the complexity of the composite tissue, where the mechanical behavior and indeed the nutrition of myocytes is intimately related to the extracellular space. Thus, the relevance of such findings to the intact myocardium remains to be elucidated.

To shed more light on this topic in the intact myocardium, this study was undertaken in 14-week-old untreated spontaneously hypertensive rats (n=11) and normotensive Wistar-Kyoto rats (n=9), we found 1) normalization of blood pressure (p<0.005) and complete regression of LVH (p<0.005) in the SHI group and no significant blood pressure or LVH reduction in the SLO group, 2) complete regression of morphometrically determined myocardial interstitial and perivascular fibrosis in SHI and SLO groups (p<0.025) associated with normalization of diastolic stiffness, measured in the isolated heart (p<0.025), and 3) regression of medial wall thickening of intramyocardial coronary arteries only in the SHI group (p<0.005), accompanied by a normalization of coronary vasodilator reserve to adenosine (p<0.005). Thus, interstitial fibrosis and not LVH is responsible for abnormal myocardial diastolic stiffness, whereas medial wall thickening of intramyocardial resistance vessels, influenced by arterial pressure, is associated with impaired coronary reserve. (Circulation Research 1991;69:107–115)
old male, spontaneously hypertensive rats (SHR), in which LVH and the adverse remodeling of the extracellular space are known to be associated with abnormal myocardial stiffness\(^\text{14}\) and impaired coronary vasodilator reserve.\(^\text{8}\) Our objectives were to dissociate each of the following: 1) LVH from the structural remodeling of the nonmyocyte compartment, 2) the interstitial and perivascular fibrosis from medial thickening of intramural resistance vessels, and 3) abnormalities in myocardial diastolic stiffness from impaired coronary reserve. In achieving these objectives, we felt it would be possible to determine the relative contribution of myocyte and nonmyocyte compartments on these functional abnormalities and therefore to derive more meaningful structure–function correlations. It has been shown that any given dose of an angiotensin converting enzyme inhibitor will inhibit the renin-angiotensin-aldosterone system in different tissues to a varying degree.\(^\text{15}\) Therefore, we used the angiotensin converting enzyme inhibitor lisinopril in two different doses: a low dose, which is known to reduce arterial pressure and almost completely inhibit plasma angiotensin converting enzyme activity in renovascular hypertension and which has no effect on blood pressure in SHR,\(^\text{16}\) and a high dose, which is necessary to normalize arterial pressure in SHR, in which the vascular tissue renin-angiotensin system is considered to play a major role in development and maintenance of hypertension.\(^\text{17}\)

Materials and Methods

Experimental Models

Age-matched, male SHR and their genetic controls, Wistar-Kyoto (WKY) rats, were obtained from Sasco Inc., Omaha, Neb. This strain of SHR showed an early development of hypertension (at 6 weeks of age) and reached a steady-state elevated blood pressure at 12 weeks of age; WKY rats were normotensive. The rats, weighing 265–325 g at the onset of the study, were studied in the following manner: 1) 14-week-old SHR (SHR\(^\text{14}\), \(n=14\)) and age-matched WKY rats (WKY\(^\text{14}\), \(n=13\)) were killed for physiological and morphological studies; 2) untreated 14-week-old SHR (SHR\(^\text{26}\), \(n=11\)) and WKY rats (WKY\(^\text{26}\), \(n=9\)) were followed for 12 weeks and killed at 26 weeks of age; 3) 14-week-old SHR were treated with a daily dose of lisinopril (2.5 mg/kg) in their drinking water for 12 weeks (SLO, \(n=11\)) and killed at 26 weeks of age; and 4) 14-week-old SHR and WKY rats were treated with a larger oral daily dose of lisinopril (20 mg/kg) in their drinking water for 12 weeks (SHI, \(n=9\), and WHI, \(n=3\), respectively) and killed at 26 weeks of age.

Weekly blood pressure determinations were obtained in all rats by the standard tail-cuff method\(^\text{18}\) to ascertain the responses in arterial pressure to each dose of lisinopril. After 1 and 9 weeks, lisinopril could be reduced to 1.25 mg/kg/day and 0.75 mg/kg/day, respectively, in the low dose–treated group. In the high dose–treated groups, drug dosage adjustments were performed at weeks 3 and 9 with reductions to 15 and 10 mg/kg/day, respectively. All rats were given standard rat chow and water ad libitum.

Physiological Studies

The rats were anesthetized (50 mg/kg i.p. methohexitol), and a carotid artery was cannulated. Arterial pressure was recorded in the lightly anesthetized state. After additional anesthesia, the rats were intubated and mechanically ventilated. The chest was opened by a median sternotomy, and the heart and lungs were removed en bloc. Within seconds, the ascending aorta was cannulated, and retrograde perfusion with 37°C crystalloid (modified Krebs-Henseleit) perfusate was established as previously reported.\(^\text{2}\) The pulmonary artery was cannulated, and coronary venous flow was measured by a Doppler flow probe (Transonics Systems, Inc., Ithaca, N.Y.). A balloon was positioned in the left ventricle (LV) via the mitral orifice and secured at the apex, which was punctured to permit the egress of thebesian drainage. The other end of the balloon was fixed to a short catheter whose outer diameter approximated that of the mitral annulus, thereby preventing regurgitation of the balloon. The catheter was connected via a stopcock to a syringe (to allow volume changes) and to a Statham pressure transducer (Gould-Statham, Oxnard, Calif.). Coronary perfusion pressure was optimized,\(^\text{2}\) and atrial pacing was used to maintain heart rate at 200 beats/min.

Steady-state LV pressure was recorded from isovolumetrically beating hearts during increments (0.02 ml) in balloon volume over the LV end-diastolic pressure range of 0–25 mm Hg. To assess myocardial stiffness for hearts of different LV weight and size, stress (\(\sigma\), dyne/cm\(^2\)), tangent elastic modulus (E, dyne/cm\(^2\)), and strain (\(\varepsilon\)) for the midwall at the equator of the LV were calculated by assuming spheroidal geometry and considering the midwall equatorial region as representative for the remaining myocardium:\(^\text{19–21}\):

\[
\sigma = \frac{V \times P}{W} \times \left(1 + \frac{4(V+W)}{[V^{1/3}+(V+W)^{1/3}]^2}\right)
\]

\[
E = 3\{V \times P \times W - \sigma + [\sigma V + (W \times \sigma - V \times P)] / W(V+W) + \sigma P \times dP/dV\} / [V^{1/3}+(V+W)^{1/3}]\]

\[
[V^{-2/3}+(V+W)^{-2/3}]
\]

\[
\varepsilon = \ln (L/Lo)
\]

where \(V\) is chamber volume (ml), \(L\) is midwall equatorial circumference (cm), \(Lo\) is circumference at end-diastolic wall stress of 0 dyne/cm\(^2\), \(W\) is left ventricular wall volume (=0.943 ml\times LV weight in grams), and \(P\) is end-diastolic pressure (dyne/cm\(^2\)=7.5\times10^{-4} \text{ mm Hg}). Myocardial diastolic stiffness was calculated using the stiffness constant (\(k\),
dimensionless), that is, the slope of the linear relation between $E$ and $\sigma$.22

Baseline coronary flow measurements were obtained at a perfusion pressure of 100 mm Hg, a heart rate of 200 beats/min, and an LV volume that coincided with an end-diastolic pressure of 0 mm Hg. A coronary perfusion pressure of 100 mm Hg was chosen, since the range of pressures associated with the autoregulated plateau in coronary blood flow included this pressure in both hypertensive and nonhypertensive experimental groups. After baseline measurements, mean coronary blood flow under maximal coronary vasodilation to adenosine (800 $\mu$g/min) was assessed for the same coronary perfusion pressure, heart rate, and filling pressure. The dose of adenosine selected was shown to abolish the hyperemic response to a 10-second occlusion of the perfusate in all hearts. Coronary flow was normalized to total ventricular weight (ml/min/g), and minimal coronary vascular resistance was calculated using the ratio of coronary perfusion pressure and coronary flow for the adenosine state (mm Hg/ml/min/g), assuming zero downstream pressure. The total duration of the physiological study was 30 minutes or less.

After the coronary flow measurements were obtained during adenosine-mediated maximal vasodilation, the hearts were immediately perfusion-fixed at 100 mm Hg with buffered glutaraldehyde (2.5%, pH 7.40) for 15 minutes. The atria and great vessels were trimmed away, and the ventricles (LV plus septum) were separated and weighed. LV dry weights were obtained by lyophilization (2 hours) and incubation of the LV myocardium in a vacuum oven at 55°C until constant weight was reached (2 hours).

**Morphology and Morphometry**

Entire coronal sections of the LV, obtained from its equator, were prepared for light microscopy as previously reported.2 Hematoxylin/eosin and the collagen-specific stain picrosirius (Sirius Red F3BA, Pfaltz & Bauer, Stamford, Conn.) were used on 5-µm-thick, paraffin-embedded sections.

Intersetital collagen volume fraction was determined by quantitative morphometry of the picrosirius-stained sections using an automated image analyzer (Quantimet 520, Cambridge Laboratories, Inc., Cambridge, Mass.). Each section was placed in a projection microscope ($\times40$). Based on their gray levels and the black appearance of collagen fibers in direct light, the images were digitized. Collagen volume fraction was calculated as the sum of all connective tissue areas of the entire coronal section, divided by the sum of all connective tissue and muscle areas in all fields of the section. Perivascular collagen was excluded from this measurement. We and others24 have previously shown that total collagen volume fraction (including perivascular collagen), as determined by this morphometric approach, is closely related to hydroxyproline concentration of the LV.

The ratio of perivascular collagen area to vessel luminal area for intramural resistance vessels was also determined with the use of the computerized image analyzer in picrosirius-stained sections. For each LV section, approximately 10 vessels were found to be cut cross-sectionally; each was examined. All collagen surrounding an intramyocardial coronary artery was considered perivascular collagen. The focal accumulation of perivascular collagen was clearly distinguishable from interstitial collagen. The area of perivascular collagen was normalized to the vessel luminal area, since a positive correlation exists between perivascular collagen area and vessel luminal area.

Average medial wall thickness of intramyocardial coronary arteries, ranging in diameter from 15 to 150 µm, was calculated as $[(VA+MA)/\pi]^{1/2}-(VA/\pi)^{1/2}$, where VA is vessel luminal area and MA is medial area. All cross-sectioned LV resistance vessels, including arterioles and small media-containing coronary arteries, were analyzed using the computerized image analyzer.

**Statistical Analysis**

All data are expressed as mean±SEM and were compared by one-way analysis of variance (ANOVA). If the omnibus hypothesis was rejected, post hoc pairwise comparisons using $t$ statistics were performed. Before initiating the study, we elected to address the following seven comparisons: SHR14/ WKY14, SHR26/WKY26, SLO/WKY26, SHI/WKY26, WHI/WKY26, SLO/SHR26, and SHI/SHR26. These multiple comparisons were corrected according to the method of Bonferroni: $p'=p/k$, where $k$ is the number of comparisons and $p'$ is the probability of error before the Bonferroni correction. Thus, the reported $p$ values are corrected $p$ values according to the method of Bonferroni. Comparisons of the $E$–$\sigma$ relations between the different experimental groups were performed by multiple regression analysis. The following sequential procedure was applied: 1) ANOVA and 2) method of dummy variables to test for intercept and slope differences.25 Regression lines are shown for each group with 95% confidence limits. Significance was taken to be $p<0.05$.

**Results**

**Hemodynamics and Hypertrophy**

In SHR14, arterial hypertension was present (Figure 1), and systolic blood pressure averaged 221±6 mm Hg compared with 129±7 mm Hg in WKY14 ($p<0.005$). In SHR26, hypertension was stable throughout the 12-week observation period. At 26 weeks of age, systolic blood pressure was 218±12 mm Hg in SHR26, compared with 124±4 mm Hg in WKY26 ($p<0.005$). SHR14 had established LVH, expressed as either a significantly increased LV to right ventricular (RV) weight ratio (Figure 2) or LV weight normalized to body weight (Table 1). RV weight normalized to body weight was no different.
between the different groups. In SHR26, the same degree of LVH (LV/RV weight ratio of 5.2±0.2 in SHR26 versus 3.7±0.2 in WKY26, p<0.005) was present as in SHR14.

In the SLO group, a comparable degree of hypertension (systolic blood pressure of 202±12 mm Hg at 26 weeks of age, p<0.005 versus WKY26) and hypertrophy (LV/RV weight ratio of 4.7±0.2, p<0.005 versus WKY26) was found; that is, there was no significant difference in systolic blood pressure and LV/RV weight ratio or LV weight normalized to body weight between groups SLO and SHR26. In contrast, in the SHI group, arterial systolic pressure was significantly reduced throughout the whole observation period (121±12 mm Hg at 26 weeks of age) and was not significantly different from the WKY26 group. The ratio of ventricular weights (3.7±0.1) and the normal LV/body weight ratio in SHI indicated that high-dose lisinopril-treated rats had no LVH. No treatment effect of lisinopril on blood pressure or LV mass was found in the WHI group: systolic blood pressure of 128±1 mm Hg at 26 weeks of age; LV/body weight ratio of 2.4±0.1 mg/g.

LV wet to dry weight ratios did not differ significantly between all groups (Table 1), indicating that myocardial edema was not present among the different experimental groups.

**Myocardial Fibrosis and Diastolic Stiffness**

The interstitial collagen volume fraction of the LV was significantly increased in 14- and 26-week-old untreated SHR compared with age-matched WKY rats (Table 2). Interstitial fibrosis was completely regressed with both high- and low-dose lisinopril, and no significant difference between SHI or SLO compared with WKY26 was found. Similarly, perivascular collagen area normalized to vessel luminal area (Figure 3) was significantly (p<0.05) increased in 14- and 26-week-old untreated SHR (SHR26: 1.4±0.2) compared with age-matched WKY rats (WKY26: 0.6±0.1). This perivascular accumulation of collagen could be restored to normal in the SHI (0.4±0.1) and

- **Figure 1.** Graph showing systolic blood pressure (SBP) in the four experimental groups: untreated spontaneously hypertensive rats (SHR), spontaneously hypertensive rats treated with low doses of lisinopril (SLO), spontaneously hypertensive rats treated with high doses of lisinopril (SHI), normotensive Wistar-Kyoto control rats (WKY). After initiation of treatment at 14 weeks of age, systolic blood pressure was normalized in SHI (no significant difference between SHI and WKY) and remained hypertensive in SLO (no significant change in comparison with SHR) throughout the observation period of 12 weeks.

- **Figure 2.** Bar graph showing left ventricular/right ventricular (LV/RV) weight ratio, which is a body weight–independent parameter of LV hypertrophy in spontaneously hypertensive rats at 14 and 26 weeks of age (SHR14 and SHR26, respectively), in 14- and 26-week-old normotensive Wistar-Kyoto rats (WKY14 and WKY26, respectively) and spontaneously hypertensive rats treated with either low or high doses of lisinopril (SLO and SHI, respectively). In SHI, complete regression of LV hypertrophy was found. In contrast, no significant change in the LV/RV weight ratio was seen in SLO compared with untreated SHR.

- **Figure 3.** Bar graph showing perivascular collagen area of intramyocardial coronary arteries normalized to vessel luminal area (PVCA/VA). Perivascular collagen was significantly increased in untreated 14- and 26-week-old spontaneously hypertensive rats (SHR14 and SHR26, respectively). In spontaneously hypertensive rats treated with either low or high doses of lisinopril (SLO and SHI, respectively), perivascular collagen was restored to levels seen in 14- and 26-week-old normotensive Wistar-Kyoto rats (WKY14 and WKY26, respectively).
SLO (0.5±0.1) groups after 12 weeks of losinopril treatment. Parallel to the interstitial and perivascular fibrosis, the slope of the linear relation between tangential elastic modulus and end-diastolic wall stress of the LV, that is, myocardial diastolic stiffness, was significantly (p<0.05) increased in 14- and 26-week-old untreated SHR compared with age-matched WKY rats (Figure 4) and could be restored to normal with either high- or low-dose losinopril; that is, the stiffness constant in the SHI and SLO groups was no different from that in the WKY26 group (Table 1). Twelve weeks of losinopril in the WHI group did not alter collagen volume fraction (2.8±0.4%) and diastolic stiffness (13.9±0.7).

**Medial Coronary Artery Thickening and Coronary Reserve**

Medial wall thickness of intramural coronary arteries after maximal adenosine-mediated vasodilatation was significantly (p<0.005) increased in 14- and 26-week-old untreated SHR compared with age-matched WKY rats and was significantly (p<0.005) reduced with high-dose losinopril, whereas it remained significantly (p<0.01) increased in the SLO group (Table 2). The difference in medial coronary wall thickness between SHR26 and SLO was not significant. Minimal coronary vascular resistance was significantly (p<0.005) elevated in SHR14, SHR26, and SLO. It could be normalized by high-dose losinopril (Figure 5). Accordingly, coronary reserve, as defined by the mean coronary blood flow normalized to ventricular weight after maximal vasodilatation to adenosine, was significantly (p<0.005) reduced in SHR14, SHR26, and SLO compared with age-matched WKY rats and could be restored to normal (p<0.005) after high-dose losinopril (Table 1). Low-dose losinopril did not alter abnormal coronary reserve or vascular resistance, and high doses of this angiotensin converting enzyme inhibitor did not influence medial coronary wall thickness (7.5±0.5 μm) or coronary reserve (27.0±1.4 ml/min) in the WHI group.

**Discussion**

The myocardium is composed of different cell types. Cardiac myocytes are the largest tissue fraction and account for 75% of the total volume of the myocardium. Myocytes, however, form only one third of all cells.26 The remaining two thirds include fibroblasts, vascular smooth muscle cells, and endothelial cells. The growth of these nonmyocyte cells can lead to a structural remodeling of the myocardium, whether or not there is associated myocyte hypertrophy. One has only to consider the structural remodeling of the coronary vasculature that follows inflammation to recognize that this is true.

In examining the remodeling of the nonmyocyte compartment that is found in the hypertension associated with unilateral renal ischemia or chronic aldosterone administration, where either plasma angiotensin II and/or plasma aldosterone are elevated, we found an interstitial and perivascular fibrosis of intramural vessels in both the normotensive, nonhypertrophied RV and in the pressure-overloaded, hypertrophied LV.4 This was not the case with the non-renin-dependent hypertension and LVH that accompanied abdominal aorta banding.

**Table 1. Hemodynamics and Left Ventricular Hypertrophy in Rats**

<table>
<thead>
<tr>
<th></th>
<th>WKY14</th>
<th>SHR14</th>
<th>SHR26</th>
<th>SLO</th>
<th>SHI</th>
<th>WKY26</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV/BW (mg/g)</td>
<td>2.6±0.1*</td>
<td>3.3±0.1*</td>
<td>3.2±0.1*</td>
<td>2.9±0.1*</td>
<td>2.3±0.1</td>
<td>2.5±0.1</td>
</tr>
<tr>
<td>RV/BW (mg/g)</td>
<td>0.63±0.02</td>
<td>0.64±0.02</td>
<td>0.63±0.03</td>
<td>0.60±0.01</td>
<td>0.60±0.01</td>
<td>0.64±0.03</td>
</tr>
<tr>
<td>W/DW</td>
<td>3.81±0.20</td>
<td>3.90±0.18</td>
<td>3.88±0.12</td>
<td>3.95±0.12</td>
<td>4.01±0.25</td>
<td>4.09±0.22</td>
</tr>
<tr>
<td>k</td>
<td>15.5±0.09</td>
<td>20.6±0.8t</td>
<td>19.5±0.9†</td>
<td>14.2±1.1</td>
<td>13.7±1.3</td>
<td>13.8±2.2</td>
</tr>
<tr>
<td>CBFmax (ml/min)</td>
<td>26.2±0.6</td>
<td>19.1±3.8t</td>
<td>17.6±1.2*</td>
<td>17.8±1.7*</td>
<td>24.6±2.3</td>
<td>29.7±1.2</td>
</tr>
<tr>
<td>CBFmax/VW (ml/min/g)</td>
<td>22.1±2.2</td>
<td>14.5±1.4†</td>
<td>12.3±0.9*</td>
<td>13.6±1.5†</td>
<td>26.0±1.4</td>
<td>21.8±2.2</td>
</tr>
</tbody>
</table>

Values are mean±SEM. WKY14, 14-week-old normotensive Wistar-Kyoto rats; SHR14, 14-week-old spontaneously hypertensive rats; SHR26, 26-week-old spontaneously hypertensive rats; SLO, spontaneously hypertensive rats treated with low-dose losinopril; SHI, spontaneously hypertensive rats treated with high-dose losinopril; WKY26, 26-week-old normotensive Wistar-Kyoto rats; LV/BW, left ventricular weight normalized to body weight; RV/BW, right ventricular weight normalized to body weight; W/DW, wet to dry weight ratio of the left ventricle; k, diastolic stiffness constant (dimensionless); CBFmax, mean coronary blood flow during maximal vasodilatation; CBFmax/VW, CBFmax normalized to total ventricular weight.

*tp<0.05, tp<0.025, tp<0.005 vs. corresponding value for age-matched WKY rats.

**Table 2. Left Ventricular Morphometry in Rats**

<table>
<thead>
<tr>
<th></th>
<th>WKY14</th>
<th>SHR14</th>
<th>SHR26</th>
<th>SLO</th>
<th>SHI</th>
<th>WKY26</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVF (%)</td>
<td>3.3±0.6</td>
<td>5.4±1.0*</td>
<td>7.0±1.3†</td>
<td>2.8±0.3</td>
<td>3.2±0.3</td>
<td>2.8±0.5</td>
</tr>
<tr>
<td>h (μm)</td>
<td>7.7±0.3</td>
<td>9.7±0.7*</td>
<td>12.3±0.62</td>
<td>9.4±0.3*</td>
<td>7.4±0.5</td>
<td>7.4±0.4</td>
</tr>
</tbody>
</table>

Values are mean±SEM. WKY14, 14-week-old normotensive Wistar-Kyoto rats; SHR14, 14-week-old spontaneously hypertensive rats; SHR26, 26-week-old spontaneously hypertensive rats; SLO, spontaneously hypertensive rats treated with low-dose losinopril; SHI, spontaneously hypertensive rats treated with high-dose losinopril; WKY26, 26-week-old normotensive Wistar-Kyoto rats; CVF, interstitial collagen volume fraction; h, medial wall thickness of maximally dilated intramural coronary arteries.

*tp<0.05, tp<0.025, tp<0.005 vs. corresponding value for age-matched WKY rats.
below the renal arteries, where plasma angiotensin II and plasma aldosterone remained normal and the interstitium and collagen volume fraction in each ventricle was no different from controls. This led us to suggest that circulating hormones (i.e., the renin-angiotensin-aldosterone system), not hemodynamic factors, mediate cardiac fibroblast growth with resultant collagen accumulation. Whether this is a direct or indirect response to these hormones is presently unclear. Nevertheless, in having separate controls for myocyte and nonmyocyte cell growth, it is possible to dissociate the consequences of their growth, including fibrosis, medial thickening of intramural coronary arteries, and myocyte hypertrophy in the intact animal. Moreover, one can examine the functional consequence to each component of this structural remodeling. In this study we were able to dissociate LVH, fibrosis, and medial thickening of intramyocardial coronary arteries and to examine their respective influence on myocardial stiffness and coronary vascular reserve to adenosine in SHR with established hypertension and LVH.

Our findings indicate that the accumulation of collagen within the interstitium of the hypertrophied LV in 14-week-old rats with genetic hypertension is accompanied by abnormal diastolic myocardial stiffness. The ability to detect changes in myocardial fibrosis and diastolic stiffness in these young SHR
compared with their genetic controls, WKY rats, depends on the strain of SHR (i.e., a strain of rats with an early development of hypertension and LVH) and the sensitivity of the methods used to assess fibrosis and stiffness. In this study, 14-week-old SHR had been hypertensive for 8 weeks and, as a result, had established LVH. We chose young adult rats for this study, since we did not want the fibrosis of aging to confound our analysis.27,28 Several other groups29,30 who examined SHR at a comparable age reported myocardial collagen concentration and collagen synthesis to be elevated when compared with WKY rats. Other investigators,27 using less sensitive methods, found only a slight increase in myocardial fibrosis in these animals. With low-dose (2.5 mg/kg/day) oral lisinopril and further reductions to 0.75 mg/kg/day, it was possible to regress myocardial fibrosis without a significant reduction in myocardial mass. Under these conditions, myocardial stiffness decreased to values seen in normal genetic normotensive controls. These findings support our view2,6 that the accumulation of collagen is a major determinant of myocardial stiffness.

We have chosen a well-established calculation of stress19–21 that assumes a thick-walled ventricle31 with spherical geometry. This calculation of stress tends to underestimate measured values, whereas the assumption of an ellipsoid would have overestimated wall stress.32 There are other potential errors in the calculation of myocardial stiffness in that we assumed isotropic behavior and homogeneity in myocardial structure. Since interstitial collagen fibers of the myocardial collagen network are distributed diffusely within the myocardium, a gross inhomogeneity in collagen accumulation is not likely. We measured LV volume and pressure in the working heart, whereas others33 obtained pressure–volume relations in the postmortem potassium chloride–arrested heart. These different techniques and the use of different strains of rats of contrasting age may explain the variability in stiffness constant between these studies and that reported herein.

Focusing on the association between collagen and diastolic stiffness in the intact myocardium, others have reached similar conclusions. O’Brien and Moore34 found that with collagenase-mediated collagen degradation the distensibility of the rabbit LV was increased; this was not the case after trypsin or elastase digestion. Bing et al.35 found that when rats with aortic banding were pretreated with the lathyrogen β-aminopropionitrile, the inhibition of collagen cross-linking prevented the abnormal rise in resting tension seen in isolated cardiac muscle strips obtained from the hypertrophied LV. In using captopril pretreatment in rats with renovascular hypertension, Jalil et al.11 were able to attenuate the rise in myocardial collagen concentration and thereby prevent the appearance of abnormal diastolic stiffness in the rat LV. Large doses (20 mg/kg/day) of lisinopril were associated with a regression in fibrosis, normalization of arterial pressure, and a restoration in myocardial mass and distensibility to values seen in age- and sex-matched WKY rats. The fact that LVH without fibrosis was seen after low doses of lisinopril, which returns myocardial stiffness to normal, suggests that LVH alone does not alter myocardial stiffness. Similar conclusions have been reached by Holubarsch and coworkers,36 who found that myocardial collagen concentration remains normal in the LVH that accompanies hyperthyroidism.

The mechanism by which lisinopril, an angiotensin converting enzyme inhibitor, might decrease myocardial collagen is yet unexplored and needs to be addressed in future studies that include in vivo experiments and culture of cardiac fibroblasts. Since angiotensin II is known to have mitogenic effects on 3T3 mouse cells,36 the interaction between the circulating and myocardial tissue renin-angiotensin-aldosterone system and fibroblast proliferation needs to be examined, given the fact that the messenger RNA for the major fibrillar collagens of the myocardium, collagen types I and III, is found in cardiac fibroblasts.37

A cautionary note should be raised regarding the importance of collagen concentration on the mechanical behavior of the intact myocardium. Previous studies from this laboratory2,3,6,38 have demonstrated that it is not merely the accumulation of collagen but also the configuration, location, and alignment of fibrillar collagen with respect to cardiac muscle that will determine myocardial stiffness. Moreover, in a composite tissue, like the myocardium, the element having the greatest stiffness constant (e.g., collagen) will have the greatest impact on its mechanical behavior.

The results of the present study also suggest that abnormal stiffness is not related to the presence of myocardial ischemia. We were able to demonstrate that, in the low-dose lisinopril–treated rats, interstitial and perivascular fibrosis regressed and myocardial stiffness was restored to normal, whereas the abnormal medial thickness of intramyocardial coronary arteries and impaired coronary vasodilator reserve remained in the hypertrophied LV. This suggests that ischemia is not likely to explain our findings. Since LV wet/dry weight ratios did not differ among the experimental groups, myocardial edema was excluded as a possible reason for abnormal stiffness.

Abnormal coronary vasodilator response to adenosine was present in untreated 14-week-old SHR, confirming the findings of others.8 Whether this abnormal vasomotor reactivity of the intramural resistance vessels was related to the abnormal accumulation of collagen in their adventitia or the growth of vascular smooth muscle has been unclear. In removing the perivascular fibrosis with low-dose lisinopril in SHR, while medial thickening remained, we found a persistent defect in coronary reserve. It was not until there was a regression in medial thickness that coronary vasodilator reserve to adenosine attained levels seen in genetic controls. Thus, it would appear
that vascular smooth muscle cell growth and its consequent thickening of the tunica media is responsible for the impaired vasomotor reactivity of intramyocardial coronary arteries. Furthermore, the fact that medial thickness did not return to control values until hypertension was controlled by high-dose lisinopril suggests that elevations in arterial pressure, or coronary perfusion pressure, may be responsible for vascular smooth muscle cell growth. When we treated WKY normotensive control rats with lisinopril, we found no direct lisinopril effect on medial wall thickness of intramyocardial resistance vessels. However, we cannot exclude the possibility that vascular smooth muscle cell growth in SHR is partly controlled by the vascular tissue renin-angiotensin system and that this tissue system is affected only by high-dose lisinopril. We did not distinguish between hyperplastic or hypertrophic coronary vascular smooth muscle cell growth in this study. In the rat aorta and larger arteries, Owens and Reidy have concluded that in slowly developing hypertension smooth muscle cell growth is hypertrophic with increased hyperplody, whereas with acute hypertension that accompanies aorta banding hyperplasia is the primary growth response. Hence, the same cell may respond differently to growth factors, and the nature of these trophic factors may depend on associated pathophysiological factors, such as altered endothelial cell permeability. The findings of our study further support the view of Cooper et al and previous findings from this laboratory that hemodynamic factors are the major determinants of cardiac myocyte growth. We did not achieve a regression in LH1 until LV systolic pressure had been reduced by high-dose lisinopril.

Thus, in genetic hypertension, diastolic myocardial stiffness appears to be closely related to the remodeling of the collagen network within the LV myocardium, whereas coronary vasodilator reserve appears to be dependent on the medial wall thickness of intramyocardial resistance vessels.

Acknowledgments

The authors wish to thank Arthur Rone, Yuqiu Guo, and Luba Verlinsky for their technical assistance and Merck Sharp & Dohme, Inc., Munich, for their support.

References

1. Thiedemann KU, Holubarsch C, Medugorac I, Jacob R: Connective tissue content and myocardial stiffness in pressure overload hypertrophy: A combined study of morphologic, morphometric, biochemical and mechanical parameters. Basic Res Cardiol 1983;78:140–155

KEY WORDS * myocardial stiffness * fibrosis * coronary reserve * coronary artery remodeling * genetic hypertension * lisinopril * spontaneously hypertensive rats
Impaired diastolic function and coronary reserve in genetic hypertension. Role of interstitial fibrosis and medial thickening of intramyocardial coronary arteries.

C G Brilla, J S Janicki and K T Weber

do: 10.1161/01.RES.69.1.107

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/69/1/107

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/