Hyperhomocysteinemia leads to adverse cardiac remodeling in hypertensive rats

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Hyperhomocysteinemia (Hhe), linked to cardiovascular disease by epidemiological studies, may be an important factor in adverse cardiac remodeling in hypertension. Specifically, convergence of myocardial and vascular alterations promoted by Hhe and hypertension may exacerbate cardiac remodeling and myocardial dysfunction. We studied male spontaneously hypertensive rats fed one of three diets: control, intermediate Hhe inducing, or severe Hhe inducing. After 10 wk of dietary intervention, cardiac function was assessed in vitro, and cardiac and coronary arteriolar remodeling were monitored by histomorphometric, immunohistochemical, and biochemical techniques. Results showed that Hhe induced diastolic dysfunction, as characterized by the diastolic pressure-volume curve, without significant changes in baseline systolic function. Perivascular collagen levels were increased by Hhe, and there was an increase in left ventricular hydroxyproline levels. Myocyte size was not affected. Coronary arteriolar wall thickness increased with Hhe due to smooth muscle hyperplasia. Mast cells increased in parallel with Hhe and collagen accumulation. In summary, 10 wk of Hhe induced myocardial collagen deposition, and diastolic dysfunction in hypertensive rats.

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**MATERIALS AND METHODS**

*Animals.* All procedures in this study were approved by the Institutional Animal Care and Use Committee of the University of Arkansas for Medical Sciences. Three-month-old male SHRs (300–325 g) were purchased from Harlan Sprague Dawley (Indianapolis, IN) and maintained in our institutional Division of Laboratory Animal Medicine facility on a 12:12-h light-dark cycle with free access to chow and water. After 7–10 days of acclimatization to this facility, the animals were randomized into three groups.

Animals in each group were then allowed free access to one of three purified amino acid diets (Harlan Teklad, Indianapolis, IN) for 10 wk: control, intermediate Hhe (IH) inducing, or severe Hhe (SH) inducing. The control and SH diets have been described by others (30). The IH diet was designed by supplementing the control diet with homocystine, the disulfide form of homocysteine (9 g/kg), and the SH diet was further supplemented with folate, choline, and methionine in the form of homocysteine (9 g/kg). Differences among the diets are shown in Table 1. The IH and SH diets were designed to produce intermediate (30–100 μmol/l) and severe (>100 μmol/l) Hhe, respectively, in the rats.

Body weight, chow consumption, and fluid intake were monitored. Blood pressure was measured at the end of the 10-wk period by cannulation of the carotid artery under anesthesia, after which time the hearts were isolated for in vitro studies. Plasma homocysteine was measured at the end of the study.

**Langendorff-perfused hearts.** This procedure is routinely used in our laboratories (14). Hearts were isolated from rats in all preparations after 10 wk of dietary intervention and perfused via the aorta with an oxygenated Krebs-Henseleit solution (37°C) of the following composition (in mM): 118.0 NaCl, 27.1 NaHCO₃, 3.7 KCl, 1.8 CaCl₂, 1.2 MgCl₂, 1.0 KH₂PO₄, and 11.1 glucose. The flow rate was set at 7.0 ml·g heart⁻¹·min⁻¹, a value similar to that observed when flow is examined at a constant pressure of 80 mmHg; coronary pressure was monitored continuously by a Statham pressure transducer. Both atria were removed, and the ventricles were paced electrically at 250 beats/min by platinum contact electrodes positioned on the interventricular septum. A fluid-filled balloon catheter (connected to a pressure transducer) was placed in the LV to measure intraventricular pressure under isovolumic conditions, and the heart was enclosed in a humidified, temperature-controlled chamber. Contractile function was monitored by measuring the peak pressure and maximum rates of contraction and relaxation (+dP/dt max and –dP/dt max) at various preload balloon volumes (10–80 μl, a range that elicited maximum contractility in all preparations). These balloon volumes represent the volume of fluid within the balloon and do not include the volume of the balloon itself, which varied very slightly from 48 to 52 μl. In addition to the polygraph recording, all data were digitized and analyzed with the use of acquisition and analysis software (CODAS, DataQ Instruments; Akron, OH).

**Morphometric measurement of collagen volume fraction and coronary arteriolar remodeling.** Morphometric measurements were done as previously described (29). Sections (5 μm) of formalin-fixed, paraffin-embedded tissue were stained with picrosirius red to identify fibrillar collagen (13). Digital images of stained sections were acquired with a charge-coupled device camera (Hamamatsu; Bridgewater, NJ) attached to a Zeiss Axioskop microscope (Zeiss; Thornwood, NY). The integrated image analysis software packages Axiovision 1 and KS300 version 3.0 (Zeiss) were used for morphometric analysis. Perivascular collagen was measured in 50- to 200-μm-diameter vessels (10–15 vessels/section) and expressed relative to the vessel luminal area. Intersitial collagen was analyzed in 30–40 microscopic fields/section, and the percentage of collagen-stained intersitial regions relative to the total ventricular area. The arteriolar wall-to-lumen ratio was analyzed in 50- to 200-μm vessels (10–15 vessels/section).

**Hydroxyproline assay.** Hydroxyproline was assayed using a procedure described by others (5, 31). The LV apex was cut into small pieces and dried to constant weight in an oven set at 65°C. The samples were then allowed to cool to room temperature, and, after being weighed, the dried tissues were transferred into 1.5-ml Eppendorf tubes. HCl (6 N, 200 μl) was added to each tube to hydrolyze the sample by overnight incubation at 105°C. After the tubes cooled, 200 μl of 6 N NaOH were added to each tube, followed by 600 μl isopropanol, and the samples were mixed well. An equal amount of each sample (10 μl) was aliquoted from the aqueous layer into individual borosilicate glass tubes and diluted with acetate-citrate-isopropanol buffer to a final volume of 400 μl; 100 μl of fresh oxidant solution were added, and the samples were mixed and allowed a 5-min incubation period. Ehrlich’s reagent solution (1.3 ml) was added, and the samples were mixed and incubated at 60°C for 30 min. After samples were cooled, the absorbance was measured at 558 nm against a blank. The reagent blank was prepared by substituting 60 μl of buffer for the sample in the reaction mixture. Hydroxyproline concentration, expressed as micrograms per milligram of heart weight, was calculated from the absorbance reading of each sample with the use of a calibration curve prepared by assaying standard solutions.

**Immunostaining for α-smooth muscle actin.** Heart tissue was fixed in formalin and embedded in paraffin. Sections (5 μm) were prepared, and immunohistochemistry was performed using anti-smooth muscle actin (Dako; Carpinteria, CA) with appropriate controls. The image analysis system discussed above was used for morphometric analysis.

**Estimation of mast cell number.** Sections of heart were stained with toluidine blue to identify mast cells by metachromasia (26). A pathologist counted the total number of mast cells in each section in a blinded fashion.

**Statistical analysis.** Data were evaluated by ANOVA with a Student-Newman-Keuls post hoc test using SigmaStat (SPSS; Chicago, IL). The criterion for significance was a P value of <0.05. Data are reported as means ± SE.

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**Table 1. Differences among the control, IH, and SH diets**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control Diet (TD99366), g/kg</th>
<th>IH Diet (TD01059), g/kg</th>
<th>SH Diet (TD10158), g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Methionine</td>
<td>8.2</td>
<td>8.2</td>
<td>0</td>
</tr>
<tr>
<td>DL-Homocystine</td>
<td>13.0</td>
<td>9.0</td>
<td>4.5</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>13.0</td>
<td>13.0</td>
<td>13.0</td>
</tr>
<tr>
<td>AIN-93-VX</td>
<td>2.5</td>
<td>Without folate</td>
<td>2.5</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

IH and SH diets, intermediate and severe hyperhomocysteinemia-inducing diets, respectively. The vitamin mix contained (in g/kg) 0.0339 nicotinic acid, 0.0181 calcium pantothenate, 0.0079 pyridoxine HCl, 0.007 thiamine HCl, 0.0068 riboflavin, 0.0023 folic acid, 0.0005 D-biotin, 0.0226 (0.1% in mannitol) vitamin B₁₂, 0.1895 (500 IU/g) indole-3-acetylacetate, 0.009 (500,000 IU/g) vitamin A palmitate, 0.0023 (500,000 IU/g) vitamin D₃, and 0.001 vitamin K.
RESULTS

We used the SHR model of genetic hypertension to determine whether short-term Hhe exacerbates myocardial and coronary arteriolar remodeling and alters cardiac function in the hypertensive heart.

Animal model of Hhe. Table 2 shows parameter values for the three groups after 10 wk of treatment with the amino acid-defined diets detailed in Table 1. Clinically, Hhe has been classified as moderate (15–30 \( \mu \)mol/l), intermediate (30–100 \( \mu \)mol/l), or severe (>100 \( \mu \)mol/l), with normal values considered to be <15 \( \mu \)mol/l (9). Mean plasma homocysteine levels in our animal models were in the normal (control), IH, and SH range. In fact, homocysteine levels in the SH group were very high (<200 \( \mu \)mol/l), intermediate (30–100 \( \mu \)mol/l), with normal values considered to be <15 \( \mu \)mol/l (9). Mean plasma homocysteine levels in our animals were in the normal (control), IH, and SH range. In fact, homocysteine levels in the SH group were very high (<200 \( \mu \)mol/l), intermediate (30–100 \( \mu \)mol/l), with normal values considered to be <15 \( \mu \)mol/l (9).

Table 2. Parameter values in control, IH, and SH rats after 10 wk of treatment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>IH</th>
<th>SH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>380 ± 5</td>
<td>393 ± 5</td>
<td>331 ± 7*</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>1.62 ± 0.06</td>
<td>1.66 ± 0.06</td>
<td>1.32 ± 0.05*</td>
</tr>
<tr>
<td>Heart weight/body weight</td>
<td>4.26 ± 0.16</td>
<td>4.22 ± 0.16</td>
<td>4.00 ± 0.16</td>
</tr>
<tr>
<td>Systolic pressure, mmHg</td>
<td>171 ± 13</td>
<td>183 ± 7</td>
<td>153 ± 9</td>
</tr>
<tr>
<td>Diastolic pressure, mmHg</td>
<td>122 ± 6</td>
<td>131 ± 4</td>
<td>110 ± 9</td>
</tr>
<tr>
<td>Pulse pressure, mmHg</td>
<td>49 ± 9</td>
<td>52 ± 7</td>
<td>43 ± 5</td>
</tr>
<tr>
<td>Plasma homocysteine, ( \mu )mol/l</td>
<td>4.3 ± 0.4</td>
<td>47.1 ± 5.1</td>
<td>202.8 ± 12.8*</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 7 \) rats/group. *Significantly different from corresponding values in the control and IH groups; †significantly different from corresponding values in the control group.

Fig. 1. Effects of balloon volume on diastolic left intraventricular (LV) pressure (A), left intraventricular developed pressure (peak systolic – diastolic; B) and the relationship between left intraventricular diastolic and developed pressures (C) in Langendorff-perfused hearts isolated from control (●; \( n = 8 \)), intermediate hyperhomocysteinemic (IH; ○; \( n = 8 \)), and severe hyperhomocysteinemic (SH; ◦; \( n = 9 \)) male spontaneously hypertensive rats (SHRs). Hearts were perfused with oxygenated Krebs-Henseleit buffer (37°C) at a flow rate of 7.0 ml·g tissue \(^{-1}\)·min \(^{-1}\) and paced at 250 beats/min. The balloon volume was increased in 10-\( \mu \)l increments, with values being recorded after steady state was achieved. A: values for diastolic pressure were not detectable at balloon volumes <30 \( \mu \)l, data shown in Fig. 1A include only those values obtained at volumes ≥30 \( \mu \)l. Hearts from IH rats tended to have a greater diastolic pressure than those from controls at balloon volumes of 70 and 80 \( \mu \)l; however, there was no significant difference between the IH and control groups or between the IH and SH groups.

The effects of increasing balloon volume on developed pressure (peak systolic – diastolic pressure) in
each of the three groups are shown in Fig. 1B. Values in Fig. 1B are presented as a percentage of the maximum value to illustrate the observed differences. Maximum values for developed pressure did not differ among the three groups (control: 79.8 ± 9.2 mmHg, IH: 78.2 ± 5.6 mmHg, and SH: 80.4 ± 6.7 mmHg). As indicated in Fig. 1B, however, hearts from IH rats approached maximum developed pressure at lower balloon volumes compared with hearts from controls (volumes corresponding to 85% of maximum developed pressure as determined by regression analysis were 51 ± 3 μl for controls and 36 ± 4 μl for IH rats). The response of hearts from the SH group did not differ significantly from those of either the control or IH groups (volume corresponding to 85% of maximum developed pressure in the SH group was 38 ± 5 μl). Similar values were obtained when monitoring +dP/dtmax and −dP/dtmax at increasing balloon volumes. Maximum observed values for +dP/dtmax were 3,297 ± 301 mmHg/s for controls, 3,163 ± 174 mmHg/s for IH rats, and 3,283 ± 229 mmHg/s for SH rats, whereas maximum values obtained for −dP/dtmax were 1,608 ± 199 mmHg/s for controls, 1,644 ± 130 mmHg/s for IH rats, and 1,652 ± 118 mmHg/s for SH rats. Figure 1C shows the relationship between diastolic pressure and developed pressure in hearts isolated from each of the three groups. Values for developed pressure in the SH group appeared to be shifted to the right of those of controls; i.e., greater diastolic pressure was required to achieve given levels of developed pressure; however, this relationship could not be tested statistically because of the nature of the data.

 Coronary artery perfusion pressure was also monitored during the experimentation in Langendorff-perfused hearts; a constant flow rate of 7.0 ml·g heart−1·min−1 was maintained in all preparations. Hhe produced a dose-dependent increase in coronary perfusion pressure, with significant increases being observed in both the IH and SH groups (80 ± 5, 110 ± 13, and 126 ± 9 mmHg in control, IH, and SH groups, respectively).

In summary, these results show that a duration of 10 wk of Hhe in hypertensive rats is associated with a diastolic dysfunction that is most likely the result of decreased myocardial compliance because the maximum rate of relaxation (−dP/dtmax) observed during examination of the pressure-volume relationship did not vary significantly. There were no significant changes in baseline systolic function. In addition, coronary perfusion pressure was significantly increased in the IH and SH groups, indicating a possible increase in coronary vascular resistance.

Effects of IH and SH on cardiac remodeling. We examined the effects of elevated homocysteine levels on the myocyte and nonmyocyte compartments in hypertensive hearts. Mean myocyte diameter, measured at the level of the nucleus, did not vary significantly among groups (data not shown). Perivascular collagen deposition around 50- to 200-μm vessels (~10–15 vessels/section) was measured in picrosirius red-stained sections and normalized to the luminal area. As shown in Table 3, perivascular collagen was increased significantly in the IH and SH groups compared with controls. Figure 2, A–C, shows representative picrosirius red-stained sections from the three groups that demonstrate the enhanced perivascular collagen deposition. Interstitial collagen (measured in 30–35 fields/section) also tended to increase in the IH and SH groups (Fig. 2, D–F); however, significant differences were not observed (Table 3). We also measured the hydroxyproline content in LV myocardium as a marker of collagen concentration. As shown in Fig. 3, tissue hydroxyproline levels increased with Hhe (control: 3.1 ± 0.52 μg/mg, IH: 4.3 ± 0.19 μg/mg, and SH: 5.27 ± 0.43 μg/mg). Figure 2, G–I, shows representative vessels from each group after immunostaining for α-smooth muscle actin; increasing medial thickness of the vessels was observed with increasing levels of plasma homocysteine. As shown in Table 3, the wall-to-lumen ratio tended to increase in both Hhe groups, reaching statistical significance in the SH group.

We also examined the relationship of mast cells to Hhe-induced cardiac remodeling because other studies have shown an association between mast cell hyperplasia and myocardial collagen accumulation in hypertensive heart disease (25). Figure 2, J–L, shows representative toluidine blue-stained sections from each group. There was an increase in mast cell number with Hhe, which reached statistical significance in the SH group (Table 3). The distribution of mast cells was predominantly perivascular and occurred in areas of fibrous tissue accumulation.

**DISCUSSION**

We examined a novel link between Hhe and the burden of CVD. Because hypertension and Hhe are highly prevalent in the general population, the potential of adverse cardiac remodeling in hypertensive hearts by Hhe would be of great clinical importance. Our results indicate that IH and SH increase collagen levels in the hypertensive LV, cause coronary arteriolar wall thickening, and result in diastolic dysfunction. Hhe has been linked to CVD in a number of previous reports. McCully (19) reported that SH resulting from inborn errors of metabolism results in vascular disease, and the prevalence of elevated plasma homocysteine levels increases from an estimated 5% in the general population to 13–47% in patients with symptomatic vascular disease (24). Retrospective and prospective studies have also shown an association be-

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>IH</th>
<th>SH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perivascular collagen</td>
<td>1.18 ± 0.90</td>
<td>6.15 ± 0.82*</td>
<td>4.74 ± 0.82*</td>
</tr>
<tr>
<td>Intersitial collagen, %</td>
<td>3.44 ± 0.21</td>
<td>6.60 ± 3.33</td>
<td>5.28 ± 1.09</td>
</tr>
<tr>
<td>Vessel wall-to-lumen ratio</td>
<td>1.70 ± 0.36</td>
<td>2.63 ± 0.32</td>
<td>3.44 ± 0.32*</td>
</tr>
<tr>
<td>Mast cell number/section†</td>
<td>50.2 ± 8.5</td>
<td>61.0 ± 24.9</td>
<td>94.2 ± 16.9*</td>
</tr>
</tbody>
</table>

*Values are means ± SE; n = 7 rats/group. Perivascular collagen is the ratio of collagen to luminal area. *Significantly different from corresponding control values. †See Fig. 2, J–L.
between Hhe and CVD, including myocardial infarction and stroke (3, 23, 28). An interesting small clinical study by Blacher and coworkers (1), in which LV mass and plasma homocysteine levels were measured in 75 patients with end-stage renal disease undergoing hemodialysis, showed a highly significant positive correlation between echocardiographically measured LV mass index and plasma homocysteine, even after adjustment for age, sex, systolic blood pressure, and hematocrit. These data suggest that there is a relationship between Hhe and LVH.

Cardiomyocytes account for approximately one-third of the cells in the myocardium, whereas they occupy 75% of its volume (10, 41). A fibrillar collagen network forms the scaffolding that leads to coordinated myocyte contraction and myocardial force generation (39). Hyperhomocysteinemia (Hhe)-induced perivascular collagen accumulation and arteriolar wall thickening. Similarly stained sections from the control (D), IH (E), and SH (F) groups show a trend toward increases in interstitial collagen in the IH and SH groups when compared with controls. α-Smooth muscle actin immunostaining shows Hhe-induced medial thickening when comparing representative vessels from the control (G), IH (H), and SH (I) groups. Toluidine blue-stained sections of the LV from control (J), IH (K), and SH (L) groups show an increased number of mast cells with Hhe. Arrows point to mast cells. See Table 3 for total mast cell numbers per section. Original magnification was ×200 for A–F and ×100 for G–L.

Fig. 2. Representative stained heart sections. Picrosirius red-stained sections from control (A), IH (B), and SH (C) groups show hyperhomocysteinemia (Hhe)-induced perivascular collagen accumulation and arteriolar wall thickening. Similarly stained sections from the control (D), IH (E), and SH (F) groups show a trend toward increases in interstitial collagen in the IH and SH groups when compared with controls. α-Smooth muscle actin immunostaining shows Hhe-induced medial thickening when comparing representative vessels from the control (G), IH (H), and SH (I) groups. Toluidine blue-stained sections of the LV from control (J), IH (K), and SH (L) groups show an increased number of mast cells with Hhe. Arrows point to mast cells. See Table 3 for total mast cell numbers per section. Original magnification was ×200 for A–F and ×100 for G–L.

Fig. 3. Hydroxyproline content of LV myocardium isolated from control, IH, and SH groups. Data show increases in the SH group compared with controls (P = 0.003). Vertical bars represent SE.
pertension and other pressure-overload conditions, such as aortic stenosis, are characterized by a combination of myocyte hypertrophy and nonmyocyte cell proliferation with increased levels of collagen. The resultant pathological LVH is characterized by increased fibrosis disproportionate to myocyte hypertrophy (39). This leads initially to deleterious effects on diastolic function and subsequently to depressed systolic function due to interference with coordinated myocyte contraction. Hence, the amount of collagen in the myocardium seems to be a major determinant of the development of cardiac dysfunction in hypertension. The development of heart failure in the SHR model is characterized by increased expression of the precursors of types I and III collagen during the transition from stable cardiac hypertrophy to overt heart failure (2). In addition, Varo and coworkers (37) demonstrated that pathological LVH with myocardial fibrosis in the SHR model is characterized by increased LV collagen concentration, increased steady-state mRNA levels for tissue inhibitor of metalloproteinase-1 (TIMP-1), which regulates collagen degradation, and decreased activity of collagenase [matrix metalloproteinase-1 (MMP-1)], the rate-limiting step in extracellular collagen degradation (16, 40).

Structural analysis of the heart in patients with hypertension has also revealed changes in the coronary arterioles. Muhnhenke and co-workers (21) showed that arterioles in patients with hypertensive heart disease have significant increases in medial area and perivascular collagen. They also showed that this remodeling is associated with reduced coronary reserve and clinical evidence of myocardial ischemia in the absence of epicardial coronary disease. In the SHR model that we used, medial thickening and perivascular fibrosis were shown to correlate with the degree of hypertension (11). This progressive cardiac remodeling and fibrosis have been postulated to increase the diffusion distance of oxygen and nutrients for myocytes, thereby contributing to myocyte dysfunction and death. Hence, these changes could lead to progressive myocyte loss, further worsening myocardial function (22, 27).

The human cardiovascular system has been shown to be limited in its ability to metabolize homocysteine. Chen and coworkers (4) determined the activity and protein levels of two major enzymes involved in homocysteine metabolism, cystathionine β-synthase and betaine homocysteine methyltransferase, in human heart tissue from rejected donor hearts. The activities of both enzymes were minimal, indicating that the heart is limited in its ability to metabolize homocysteine via these pathways and thus depends primarily on the folate- and vitamin B12-dependent remethylation (methionine synthase) pathway to clear homocysteine. Hence, it is likely that the heart may be especially sensitive to the effects of elevated plasma homocysteine levels.

We examined the effect of Hhe in SHRs using two diets based on homocysteine (the disulfide form of homocysteine) supplementation. The diets were successful in elevating plasma homocysteine levels to the intermediate and severe ranges. The SH diet combined deficiencies of choline, methionine, and folate with homocysteine supplementation and has been used previously in studies of carotid vascular disease (30). The plasma homocysteine level seen in the SH group in the present study was greater than that documented in the previous report (30), possibly because of the longer duration of dietary treatment. As discussed below, it is possible that folate deficiency played some role in the observed cardiac effects in the SH group; however, there was no significant decrease in hemoglobin or hematocrit after the 10-wk treatment period in our study (data not shown). Nonetheless, because Hhe in the general population frequently coexists with folate, vitamin B6, or vitamin B12 deficiency, this model has close parallels to the human condition. Blood pressure after the 10 wk of treatment showed a tendency to increase in the IH group [as reported previously (20)] and a tendency to decrease in the SH group. Similarly, as indicated in Fig. 1B, the shift of the curve showing effects of intraventricular balloon volume on LV developed pressure in the IH group was partially reversed in the SH group. The absence of a “concentration dependency” in terms of the effects of homocysteine on blood pressure and the “ventricular function curve” cannot be explained by current data, but it is possibly mediated by folate/methionine/choline deficiency-induced actions in SH that antagonized the effects of Hhe.

The results of our experiments demonstrated that Hhe is associated with a significant increase in peri- vascular collagen in hypertensive hearts. Interstitial collagen levels also tended to increase in both Hhe groups relative to controls, but this did not reach statistical significance. This parallels the actions of various chemokines known to affect myocardial remodeling (e.g., angiotensin II and aldosterone), which initiate myocardial collagen deposition in the perivascular region followed by an interstitial distribution (39, 40). The interstitial collagen deposition could represent replacement fibrosis after myocyte loss (39). In addition, we demonstrated an increase in the hydroxyproline content of LV myocardium confirming that total collagen content increased with increasing plasma levels of homocysteine.

The sequence of events leading from elevated plasma homocysteine levels to increased collagen levels is a matter of speculation. Potential mechanisms include possible direct effects of homocysteine on fibroblast proliferation and function leading to increased collagen production or direct effects on collagen metabolism (35). Tyagi and coworkers (36) studied the effect of homocysteine on MMP activity in human coronary artery extracts. They showed a biphasic response, with an increase in collagenolytic activity at homocysteine concentrations <0.1 mM. In addition, homocysteine has been reported to induce TIMP-1 expression in vascular smooth muscle cells (32). Alternatively, high homocysteine levels may cause endothelial dysfunction and expression of adhesion molecules (12) followed by adhesion and activation of inflammatory cells, secre-
tion of chemokines/growth factors, altered fibroblast function, and collagen deposition. The proximate events and signaling mechanisms that influence fibroblast, endothelial, or inflammatory cell function may include oxidative stress or signaling pathways such as protein kinase C and cell cycle proteins (38).

We examined the relation of the inflammatory cell response, specifically mast cell accumulation, to Hhe-induced cardiac remodeling in hypertensive hearts. Mast cells have been correlated with the severity of fibrosis in conditions such as scleroderma, idiopathic pulmonary fibrosis, and keloids (25). Li and co-workers (17) described an association between mast cells and the amount of myocardial fibrosis in transplanted human hearts. Another study (25) examined the relationship of mast cells to cardiac remodeling in the LV of SHR and found significant increases in perivascular and interstitial collagen levels and an increased number of mast cells compared with normotensive controls.

Therapy to decrease blood pressure was associated with a decrease in both collagen accumulation and mast cell number. We observed a dose-dependent increase in mast cells in hypertensive hearts subjected to Hhe that paralleled collagen accumulation. This interesting observation suggests that mast cells may play a role in cardiac remodeling secondary to Hhe in hypertensive hearts.

Hhe also promoted diastolic dysfunction in hearts of hypertensive rats. The pressure-volume relationship was altered, showing an upward/leftward shift with increasing levels of homocysteine. Data from echocardiographic studies have shown that the LV chamber volume is decreased slightly in hearts from SH rats compared with control rats; however, this small change (diastolic diameter was decreased by ~8%, data not shown) cannot account for the observed changes in the pressure-volume curve. In contrast to the effect of Hhe on compliance, the rate of relaxation expressed as the pressure-volume curve. In contrast to the effect of Hhe on compliance, the rate of relaxation expressed as the pressure-volume curve. In contrast to the effect of Hhe on compliance, the rate of relaxation expressed as the pressure-volume curve. In contrast to the effect of Hhe on compliance, the rate of relaxation expressed as the pressure-volume curve. 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