Matrix Metalloproteinase Inhibition During the Development of Congestive Heart Failure
Effects on Left Ventricular Dimensions and Function


Abstract—The development of congestive heart failure (CHF) is associated with left ventricle (LV) dilation and myocardial remodeling. The matrix metalloproteinases (MMPs) play a significant role in extracellular remodeling, and recent studies have demonstrated increased MMP expression and activity with CHF. Whether increased MMP activity directly contributes to the LV remodeling with CHF remains unknown. Accordingly, this study examined the effects of chronic MMP inhibition (MMPi) on LV size and function during the progression of CHF. Pigs were assigned to the following groups: (1) CHF, rapid pacing for 3 weeks at 240 bpm (n = 12); (2) CHF/MMPi, rapid pacing and concomitant MMPi (PD166793, 20 mg/kg per day [n = 10]), and (3) control (n = 11). With pacing CHF, LV fractional shortening was reduced (19±1 versus 45±1%), and end-diastolic dimension increased (5.67±0.11 versus 3.55±0.05 cm), compared with baseline values (P<0.05). In the CHF/MMPi group, LV endocardial shortening increased (25±2%) and the end-diastolic dimension was reduced (4.92±0.17 cm) compared with CHF-only values (P<0.05). LV midwall shortening was reduced to a comparable degree in the CHF-only and CHF/MMPi groups. LV peak wall stress increased 3-fold with pacing CHF compared with controls and was significantly reduced in the CHF/MMPi group. LV myocardial stiffness was unchanged with CHF but was increased in the CHF/MMPi group. LV myocyte length was increased with pacing CHF compared with controls (180±3 versus 125±4 μm, P<0.05) and was reduced in the CHF/MMPi group (169±4 μm, P<0.05). Basal-state myocyte shortening velocity was reduced with pacing CHF compared with controls (33±2 versus 66±1 μm/s, P<0.05) and was unchanged in the CHF/MMPi group (31±2 μm/s). Using an ex vivo assay system, myocardial MMP activity was increased with pacing CHF and was reduced with chronic MMPi. In summary, concomitant MMPi with developing CHF limited LV dilation and reduced wall stress. These results suggest that increased myocardial MMP activity contributes to LV myocardial remodeling in developing CHF. (Circ Res. 1999;85:364-376.)

Key Words: congestive heart failure • metalloproteinases • myocyte function

The development of congestive heart failure (CHF) is accompanied by left ventricle (LV) dilation and pump dysfunction.1–3 In patients with CHF, the progressive LV dilation is associated with an increased incidence in morbidity and mortality.1–4 These clinical observations, as well as experimental studies, suggest that LV remodeling is an important contributory event in the progression to severe CHF.1–9 However, the mechanisms that contribute to the changes in LV geometry and function that occur during the CHF process remain unclear. An important constituent of the LV myocardium is the fibrillar collagen matrix, which contributes to the maintenance of LV geometry and the structural alignment of adjoining myocytes.10–13 Alterations in collagen structure and composition have been reported to occur within the LV myocardium in several cardiac disease states, which in turn may influence LV geometry.5–7,10,11,14–17 An endogenous enzyme system responsible for extracellular collagen degradation and remodeling is the matrix metalloproteinases (MMPs).18–27 Increased myocardial MMP activity has been reported to occur with the development of severe CHF, such as in cardiomyopathic disease.5,6,25–27 However, whether heightened MMP activity contributes to the LV remodeling that occurs during the evolution of the CHF process remains unknown. Accordingly, the overall goal of the present study was to determine whether chronic MMP inhibition would influence LV geometry and function in the setting of CHF.

Chronic pacing–induced tachycardia in animals causes well-defined, predictable, and progressive LV dilation, con-
tractile dysfunction, and neurohormonal system activation.5,6,14–16,28,29 These functional and neurohormonal changes are similar to the clinical spectrum of CHF.1,2 Therefore, this chronic pacing model may provide an opportunity to identify contributory events responsible for the progression of LV dilation and dysfunction that occurs with CHF. It has been previously demonstrated that the fibrillar collagen weave supporting adjacent myocytes is reduced with the development of pacing-induced CHF.5,14–16 Using in vitro proteolytic assay systems, increased LV myocardial MMP activity has been observed with the development of pacing-induced CHF.5,6 These past studies provide indirect evidence to suggest that fibrillar collagen degradation and remodeling contribute to the progressive LV dilation in this model of CHF. Accordingly, the present study was designed to test the central hypothesis that chronic interruption of myocardial MMP activity will reduce the LV dilation that invariably occurs in this model of CHF.

Materials and Methods

Dose Selection Studies

Three Yorkshire pigs (22 to 25 kg, male, Hamborne Farms, Orangeburg, SC) were chronically instrumented with an aortic vascular access port to measure arterial blood pressure in the conscious state, as described previously.5,20 After a recovery period of 7 to 10 days, the animal was returned to the laboratory for baseline studies. For these studies, the animals were sedated with diazepam (Valium, 20 mg PO, Hoffmann-La Roche) and placed in a custom-designed sling. The vascular access port was entered using a 12-gauge Huber needle (Access Technologies), and basal and resting arterial pressures and heart rate were recorded. Pressures from the fluid-filled aortic catheter were obtained using an externally calibrated transducer (Statham P23ID, Gould). After baseline studies, the pigs were then used for initial MMP inhibition dosage studies. All animals were treated and cared for in accordance with the NIH Guide for the Care and Use of Laboratory Animals (National Research Council, Washington, 1996).

The MMP inhibitor chosen for these studies was PD166793, which has a chemical formulation of (5)-2-(4′-bromo-biphenyl-4-sulfonylamino-3-methyl butyric acid) and has global MMP inhibitory activity in the 8 to 10 μmol/L range, based on in vitro assay systems (Table 1).30–32 This MMP inhibitor was also examined with respect to activity against angiotensin-converting enzyme (prepared from rabbit lung),33 neutral endopeptidase (24.11, membrane fraction from Burkitt lymphoma cell line),34 endothelin-converting enzyme (membrane fraction from CHO cells transfected with human endothelin-converting enzyme 1),35 and tumor necrosis factor-α convertase (tumor necrosis factor-α release from stimulated leukocytes; PanLabs Inc).36 Concentrations of up to 100 μmol/L of PD166793 did not exhibit inhibitory activity against these proteolytic systems (Table 1). These results demonstrated that this compound possessed no activity against other enzymatic systems that have been identified to be relevant in the CHF process.37,38

A single oral dose of 10 mg/kg PD166793 was administered in one pig, and a second pig was simultaneously administered a single 10 mg/kg dose of the MMP inhibitor intravenously. A pharmacokinetic profile was constructed using these initial data, and it was predicted that an oral delivery of 20 mg/kg per day of PD166793 would provide significant MMP plasma inhibitory activity at trough levels. Accordingly, the 3 pigs underwent 20 mg/kg QD (morning) treatment using the MMP inhibitor PD166793 for 5 days. This dosage regimen resulted in a drug plasma level that inhibited MMP activity by 100%, based on the ex vivo thiopeptilide assay.30–32

### Table 1. Protease Inhibitory Activity of the Matrix Metalloproteinase Inhibitor PD 166793

<table>
<thead>
<tr>
<th>Enzyme Classification</th>
<th>IC₅₀, μmol/L*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMPs†</td>
<td></td>
</tr>
<tr>
<td>Interstitial collagenase MMP-1</td>
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<tr>
<td>Stromelysins</td>
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</tr>
<tr>
<td>MMP-3</td>
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<tr>
<td>MMP-7</td>
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<td>Gelatinases</td>
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<td>0.047</td>
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<tr>
<td>MMP-9</td>
<td>9.900</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme</td>
<td>(NC)&gt;100μmol/L</td>
</tr>
<tr>
<td>Neutral endopeptidase</td>
<td>(NC)&gt;100μmol/L</td>
</tr>
<tr>
<td>Endothelin-converting enzyme</td>
<td>(NC)&gt;100μmol/L</td>
</tr>
<tr>
<td>TNF-α-converting enzyme</td>
<td>(NC)&gt;100μmol/L</td>
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</tbody>
</table>

TNF indicates tumor necrosis factor.

*Data expressed as concentration necessary to inhibit 50% of maximal enzyme activity.
†MMP activity assays determined using recombinant human MMP constructs of the catalytic domain and an artificial substrate assay system.
§PD166793 was not active at up to 100 μmol/L; therefore, a true IC₅₀ was not computed (NC).

Experimental Protocol and Animal Model Preparation

After the MMP inhibition dose selection studies, the effects of concomitant treatment with MMP inhibition with chronic rapid pacing were examined. Weight-matched pigs (22 to 23 kg) were randomly assigned to 3 groups, as follows: (1) rapid atrial pacing (240 bpm) for 3 weeks (n=12), (2) concomitant MMP inhibition (PD166793 20 mg/kg per day) and rapid pacing (n=10), and (3) sham controls (no pacing; n=11). The drug treatment was begun 3 days before the initiation of pacing and continued for the entire 21-day pacing protocol. The procedures used for the placement of the pacing electrode and pacemaker have been well described previously.5,14,28,30–41 Ten to fourteen days after recovery from the surgical procedure, baseline studies were performed, and the protocols described above were begun.

LV Function and Hemodynamic Measurements

Weekly measurements of LV size and function were obtained in the rapid pacing group (n=8) and in the rapid pacing and MMP inhibition group (n=7). At the conclusion of the 3-week protocol, LV function was examined in all of the pigs entered in the protocol. For these studies, the animals were brought to the laboratory, and the pacemaker was deactivated. All measurements were performed at an ambient resting heart rate within 30 to 40 minutes after pacemaker deactivation. Two-dimensional and M-mode echocardiographic studies (2.25-MHz transducer, ATL Ultramark VI) were used to image the LV from a right parasternal approach.29,40 LV endocardial fractional shortening was calculated as (end diastolic dimension–end systolic dimension)/end diastolic dimension and was expressed as a percentage. LV midwall fractional shortening was calculated at the level of the LV minor axis using methods described previously by Gaasch et al42 and Shimizu et al.43 The mean velocity of circumferential fiber shortening corrected for heart rate (Vcf(L)) was calculated using the LV echocardiographic dimension measurements and the aortic pressure trace, as described previously.44 Peak circumferential global average wall stress was computed using a spherical model of reference: σg/cm² = [PD/(h + D) × 1.36], where P=peak aortic pressure measured from the access port, D=minor axis dimension at end diastole, h=wall thickness, and 1.36 is a coefficient constant for conversion to g/cm². This stress computation was chosen because it...
reflects the LV wall stress pattern that will be most affected by the changes in LV geometry due to the myocardial remodeling process. It has been demonstrated previously that significant changes in LV geometry and loading conditions occur with pacing-induced CHF.14,15,28,29,39–41 To more carefully examine LV ejection performance in relation to changes in LV afterload, LV endocardial and midwall fractional shortening were measured after incremental increases in LV myocardial wall stress in 5 control pigs, as described previously.4,40 This approach for measuring the LV shortening-stress relation has been reviewed in detail previously.4,44 To determine LV performance in the context of LV afterload during the entire ejection phase, mean circumferential wall stress was computed.42,43 The LV shortening-mean stress points for the control pigs were subjected to linear regression, and the 95% confidence interval was computed. The LV ejection-mean stress points for each of the rapid pacing groups were then plotted with respect to these normal control relationships.

After completion of the 21-day protocol, a final set of LV function and hemodynamic measurements were performed, and plasma was collected for neurohormonal profiles and MMP inhibitor compound levels. The pigs were anesthetized as described in the previous section, and the LV and pericardium harvested. The LV was divided and processed for myocyte isolation, perfusion-fixed for histology, and flash-frozen for MMP assays. A subset of animals (control [n=4], rapid pacing [n=4], and pacing with MMP inhibition [n=3]) were instrumented for LV diastolic function studies as described in the following paragraph.

LV Stiffness Properties

The fibrillar collagen matrix has been implicated as an influence on LV myocardial stiffness properties.11,14,17,47,48 LV diastolic function has been well characterized previously in this pacing model of CHF.14,40,49–51 Because the goal of the present study was to manipulate myocardial fibrillar collagen degradative processes, indices of stiffness constant (Kc) were based on the analyses of the stress-strain relation. It has been demonstrated previously that significant changes in LV myocardial wall stress in 5 control pigs, as described previously.5,40 This approach for measuring the LV shortening-stress relation has been reviewed in detail previously.4,44 To determine LV performance in the context of LV afterload during the entire ejection phase, mean circumferential wall stress was computed.42,43 The LV shortening-mean stress points for the control pigs were subjected to linear regression, and the 95% confidence interval was computed. The LV ejection-mean stress points for each of the rapid pacing groups were then plotted with respect to these normal control relationships.

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Indices of LV diastolic function were determined by computations of the regional LV chamber stiffness constant (Kc) and myocardial stiffness constant (Km).40,48 Calculations for Kc were based on analyses of the LV end-diastolic pressure versus LV end-diastolic chamber dimension using an exponential function given by

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P = P_0 e^{KcD} \]

where P is LV end-diastolic pressure, D is chamber dimension, and A and Kc are fitting constants. The natural logarithm of this function was used to compute Kc by linear regression. Calculations of Km were based on the analyses of the stress-strain relationship.40

Neurohormonal and MMP Inhibitor Measurements

The plasma samples were assayed for renin activity, catecholamine levels, and plasma MMP inhibition levels. Plasma renin activity was determined by computing angiotensin I production using a radioimmunoassay (NEA-026, New England Nuclear). Plasma norepinephrine was measured using HPLC and normalized to pg/mL of plasma. Plasma and myocardial measurements of the MMP inhibitor PD166793 were first extracted through a chromatography column, and compound levels determined by reverse-phase HPLC.

LV Myocyte Contractile Function

Myocytes were isolated from the LV free wall using methods described previously by this laboratory.5,28,29,39,41 Briefly, the left circumflex coronary artery was perfused with a collagenase solution (0.5 mg/mL, Worthington, type II; 146 U/mg), and the liberated myocytes (5 × 10⁴ cells/mL) were resuspended in cell culture medium (MEM, Gibco Laboratories). LV myocyte contractility was examined using computer-assisted videomicroscopy.28,44 Specifically, the LV myocytes were stimulated at 1 Hz and the profiles of the contracting myocyte digitized.28,29,41 All measurements were performed using identical electrical field stimulation parameters. After baseline measurements, contractile function was also examined after a specific inotropic stimulus in each myocyte either after β-adrenergic receptor stimulation with 25 mmol/L isoproterenol (Sigma) or in the presence of 8 mmol/L extracellular Ca²⁺.

LV Myocardial Fibrillar Collagen Structure

Full-thickness sections of the perfused LV myocardium were immersed in fresh fixative overnight. Slices 4 µm in thickness were cut from the blocks and mounted on glass slides. The sections were then rehydrated and stained using the picrosirius histochemical technique.52 The sections were then digitized at a final magnification of 320× and analyzed with an image-analysis system (Sigma Scan/Image, Jandel). The percentage area of extracellular staining was computed from 15 random fields within the midmyocardium to exclude large epicardial arteries and veins and any cutting or compression artifact.14,29,48 The collected pericardial samples were fixed overnight and prepared in a similar fashion.

LV Zymographic MMP Activity

MMP Zymography

The LV myocardial samples were prepared for zymographic studies as described previously.5,6,20,28,29 LV MMP gelatinase activity and abundance were examined by substrate-specific zymography.5,6,20

To provide an internal control with respect to the zymographic activity, cell culture medium samples from a cultured human fibrosarcoma HT 1080 cell line were included.53 The zymograms were digitized, and the size-fractionated banding pattern, which indicated MMP proteolytic activity, was determined by quantified image analysis (Gel Pro Analyzer, Media Cybernetics). The lysis areas were measured by 2-dimensional integrated optical density computations and are expressed in pixels.

MMP Substrate Assay

Quantitative MMP activity was determined in crude LV myocardial extracts (which were not detergent treated or separated by electrophoresis) using gelatin as the MMP substrate. A gelatin matrix was used that was conjugated to quenched FITC (Molecular Probes). Gelatin-FITC (1 mg/mL diluted in 1% agarose, 50 mmol/L Tris-Cl, 5 mmol/L CaCl₂, and 0.02% NaN₃ [pH 7.5]) was loaded onto a 96-well plate (50 µg/well; Nalge Nune International) and allowed to polymerize (25°C, 30 minutes). LV myocardial extracts were loaded onto the wells (95 µg total protein) and incubated (37°C, 2 hours). The proteolytic release of the FITC chromogen was recorded using dual-wavelength spectrophotometry (490/570 nm, S2000 Microplate Reader, Fisher Scientific).

To confirm the concentration dependency as well as specificity of this system, serial dilutions of a purified recombinant construct of the catalytic domain for the human gelatinase, MMP-2, was used. The MMP-2 catalytic domain construct was obtained through expression in Escherichia coli using an optimized DNA codon that encodes the human MMP-2 amino acid sequence with the fibronectin binding domain deleted.52 A concentration-dependent proteolysis of the FITC-labeled gelatin substrate was observed (Figure 1, inset). Next, LV myocardial extracts (25 µg total protein) were incubated with the
gelatin substrate in the presence of the following: the serine protease inhibitor aprotinin (100 μmol/L, Miles, Inc); the metal-chelating agent EDTA (20 mmol/L, Sigma); and galardin (5 mmol/L), a previously characterized global MMP inhibitor.54,55 LV myocardial gelatinase activity was unaffected by serine protease inhibition but was reduced in a predictable fashion by EDTA. More importantly, LV myocardial gelatinase activity was decreased using the MMP inhibitor galardin. These results indicated that the proteolytic cleavage of gelatin that occurred in the presence of LV myocardial extracts was primarily due to MMP activity. Inhibitory studies were performed in triplicate.

**MMP-2 Activity by Antibody Capture Assay**

An additional series of studies was performed in which specific MMP-2 activity was measured by an antibody capture method. For this assay, purified MMP-2 or control LV myocardial extracts (50 μg total protein) were incubated on a 96-well microtiter plate in which a monoclonal MMP-2 antibody was immobilized (RPN2631, Amersham Pharmacia Biotech). The specificity and concentration dependency of this MMP-2 activity assay system was first examined using full-length MMP-2 standards (1 to 12 ng/mL) purified from a human MMP-2 catalytic domain.32 A concentration-dependent proteolysis of the FITC-labeled gelatin substrate was observed. Quantitative LV myocardial proteolytic activity was determined using a fluorescent gelatin substrate in myocardial homogenates (95 μg total protein) in the basal state, as well as after incubation with aprotinin (100 μmol/L), a serine protease inhibitor; EDTA (20 mmol/L), a metal-chelating agent; and galardin (5 mmol/L), a previously characterized global MMP inhibitor.54,55 LV myocardial gelatinase activity was unaffected by serine protease inhibition but was reduced in a predictable fashion by EDTA. More importantly, LV myocardial gelatinase activity was decreased using the MMP inhibitor galardin. These inhibitory studies were performed in triplicate.

*P*<0.05 vs basal.

**Data Analysis**

Indices of LV and myocyte function were compared among the 3 treatment groups using multiway ANOVA. For comparisons of LV function with each week of pacing, an ANOVA for repeated measures was used. If the ANOVA revealed significant differences, pairwise tests of individual group means were compared using Bonferroni probabilities. For comparisons of neurohormon al profiles and MMP activity, the Student-Neuman-Keuls test was used. The chamber and myocardial stiffness constants were compared among the 3 groups using the Mann-Whitney test. All statistical procedures were performed using the BMDP statistical software package. Results are presented as mean±SEM. Values of *P*<0.05 were considered to be statistically significant.
Results
All of the pigs entered into the rapid pacing protocol were successfully studied. Steady-state plasma levels at the conclusion of the study for the MMP inhibitor were 93±10 μmol/L, and myocardial concentrations were 15.7±2.5 μg/g. A significant linear relationship was observed between plasma and myocardial levels of the MMP inhibitor (r=0.94, P<0.05). The myocardial concentration of the MMP inhibitor was consistent for the concentration necessary to significantly inhibit myocardial MMP-2 proteolytic activity (Figure 2). The plasma levels of PD166793 obtained in the present study are consistent with levels necessary for inhibition against several MMP species (Table 1).

LV Function and Neurohormones With Rapid Pacing: Effects of Chronic MMP Inhibition
Representative LV echocardiographic recordings taken at each week of rapid pacing, with and without concomitant MMP inhibition, are shown in Figure 3. Weekly indices of LV function obtained with chronic rapid pacing and with MMP inhibition are summarized in Figure 4. In the untreated rapid pacing group, LV end-diastolic dimension and peak wall stress increased from baseline values in a time-dependent manner. These changes in LV geometry were associated with a decline in LV fractional shortening. In the rapid pacing and concomitant MMP inhibition group, the degree of LV dilation was significantly attenuated when compared with untreated rapid pacing values. This reduction in LV end-diastolic dimension was translated into a significant reduction in LV peak wall stress when compared with rapid pacing-only values. LV fractional shortening fell significantly in the rapid pacing and MMP inhibition group but remained higher than untreated rapid pacing values.

LV function and hemodynamics are summarized in Table 2 for baseline conditions (before pacemaker activation) and after 21 days of rapid pacing. After 21 days of chronic rapid pacing without treatment, ambient resting heart rate was increased and resting blood pressure decreased from baseline values. In the rapid pacing–only group, Vcf decreased by >30% from baseline values. In the rapid pacing and MMP inhibition group, resting heart rate was increased and mean arterial pressure was reduced from baseline conditions. However, resting heart rate was lower in the rapid pacing and MMP inhibition group when compared with rapid pacing–only values. In the rapid pacing and MMP inhibition group, the LV end-diastolic dimension was reduced and wall thickness was increased from untreated rapid pacing values. As a result, LV peak wall stress was significantly lower in the rapid pacing and MMP inhibition group when compared with rapid pacing–only values. LV fractional shortening increased by 44% in the rapid pacing and MMP inhibition group when compared with the untreated rapid pacing group. In the rapid pacing and MMP inhibition group, LV Vcf, was greater than the rapid pacing–only values, but this difference did not reach statistical significance. LV midwall fractional shortening was significantly reduced in the rapid pacing group and remained significantly reduced in the MMP inhibition group.

To more carefully examine the relationship between indices of LV ejection and LV afterload (wall stress), both the LV endocardial and midwall fractional shortening–mean stress relationships were determined (Figure 5). After chronic pacing, a downward and rightward shift was observed, indicating a significant decline in LV ejection performance. In the rapid pacing and MMP inhibition group, a reduction in LV wall stress occurred that resulted in a leftward shift in the LV ejection-stress relationships when compared with values from the untreated rapid pacing group. However, in the MMP inhibition group, the steady-state values for these indices of LV ejection performance remained below the 95% confidence interval obtained from normal control animals. Thus, consistent with past reports, these relatively load-insensitive indices of LV systolic performance were markedly impaired, with chronic rapid pacing. Moreover, whereas MMP inhibition during chronic rapid pacing reduced LV wall stress, the results from this analysis demonstrated persistent defects in LV myocardial contractile performance.

The chamber and myocardial stiffness constants were computed in a subset of pigs from the control, rapid pacing, and rapid pacing with MMP inhibition groups. As reported previously, these indices of LV stiffness properties were examined using the portion of the stress-strain relations in which LV pressures were comparable. In the rapid pacing group, the LV chamber stiffness constant, K, was unchanged from control values (1.7±0.3 versus 1.7±0.2). The LV myocardial stiffness constant, Kmy, was higher with rapid pacing, but this did not reach statistical significance (11.4±1.7 versus 8.9±1.2, respectively, P=0.25). In the rapid pacing and MMP inhibition group, both LV chamber and myocardial stiffness constants were increased from control values (3.1±0.5 and 18.3±2.1, respectively, P<0.05).

Plasma renin activity and norepinephrine values increased by >3-fold in the rapid pacing–only group (Table 2). These indices of neurohormonal activation were similarly increased with rapid pacing and concomitant MMP inhibition. There was no difference in LV mass/body weight in the rapid pacing group compared with sham controls (3.6±0.2 versus 3.1±0.2 g/kg), and this remained unchanged in the MMP inhibition group (3.7±0.2 g/kg, P=0.25). At necropsy, there was some observable pericardial thickening in both rapid pacing groups, but the pericardium was nonadherent to the epicardial surface. Pericardial thickness, computed from fixed and embedded sections, was increased from controls in the rapid pacing group (116±6 versus 220±53 μm, P<0.05) and was also increased in the MMP inhibition group (224±26 μm, P<0.05). No other changes in gross organ appearance or musculoskeletal morphology could be appreciated. The joints and cartilage of all extremities appeared grossly normal.

LV Myocyte Contractility With Rapid Pacing: Effects of Chronic MMP Inhibition
Myocyte contractile function was examined in >300 LV myocytes from the sham control group, after 3 weeks of chronic rapid pacing, and with rapid pacing and concomitant MMP inhibition, and the results are summarized in Table 3. LV myocyte resting length was increased in the untreated...
rapid pacing group when compared with controls. In the rapid pacing and MMP inhibition group, resting myocyte length was reduced from untreated rapid pacing values but remained significantly increased from normal control values. Steady-state myocyte contractile function was significantly reduced in the untreated rapid pacing group when compared with normal control values. In the rapid pacing and MMP inhibition group, indices of steady-state myocyte contractile function were unchanged from untreated CHF values. However, the time to peak contraction and total contraction duration were reduced from rapid pacing–only values. β-Receptor stimulation with isoproterenol increased myocyte function from basal values in all 3 groups. However, in the presence of isoproterenol, myocyte contractile function was significantly blunted in the untreated rapid pacing group and the MMP inhibition group when compared with normal control values. With inotropic stimulation, indices of active relaxation, such as the time to 50% relaxation and total contraction duration,
fraction was 10.6±0.9%, which was higher than rapid pacing–only and control values (P<0.05).

LV Myocardial Zymographic Activity With Rapid Pacing: Effects of MMP Inhibition
LV myocardial MMP zymographic activity was examined in LV myocardial extracts from the control, rapid pacing, and rapid pacing with MMP inhibition groups. Zymographic activity was increased in the rapid pacing group compared with controls (7719±665 versus 4910±854 pixels, P<0.05). In the rapid pacing and MMP inhibition group, MMP zymographic activity was increased from controls and was similar to untreated rapid pacing–only values (8583±840 pixels). During separation and electrophoresis, this in vitro assay system separates and removes any of the MMP inhibitor from the LV myocardial samples. Thus, this zymographic assay reflects total LV myocardial MMP abundance and does not reflect actual MMP activity in vivo. To more carefully determine the effects of MMP inhibition on myocardial MMP activity, gelatinase activity was quantified in crude LV myocardial homogenates in which purification and electrophoretic separation were not performed (Figure 7). LV myocardial gelatinase activity was increased in the rapid pacing group at all myocardial extract concentrations, which indicated increased myocardial MMP activity. In the MMP inhibition group, myocardial gelatinase activity was normalized, which provides evidence that the MMP inhibitor PD166793 decreased MMP activity from rapid pacing values in vivo. In an additional series of studies, gelatinolytic activity was examined in LV myocardial extracts after activation with the organomercurial p-aminophenylmercuric acetate (APMA).21,23 LV myocardial gelatinase activity after APMA activation was computed as a percentage change from unstimulated, basal values (Figure 7). In the pacing CHF group, LV myocardial gelatinase activity significantly increased from baseline. In the rapid pacing and MMP inhibition group, LV myocardial gelatinase activity after APMA stimulation was significantly blunted.

Discussion
The progression of LV dilation in patients with CHF has been demonstrated to be associated with an increased incidence of morbidity and mortality.1–4 Furthermore, experimental studies have demonstrated that an attenuation of LV dilation in the setting of CHF was associated with improved survival.5,6 The LV myocardial extracellular matrix has been implicated as playing an important role in maintaining chamber shape and myocyte alignment.10–13,56,57 Increased activity of the MMPs, an enzyme system responsible for extracellular remodeling, has been reported in both clinical and experimental forms of CHF.5,6,14–16,28,29,39–41 However, whether and to what degree MMP inhibition may influence the LV remodeling process with developing CHF remained unexplored. Chronic rapid pacing in animals has been demonstrated previously to cause LV dilation and pump dysfunction in a progressive and time-dependent manner, which invariably produces severe CHF.5,6,14–16,28,29,39–41 Accordingly, in the present study, chronic MMP inhibition was instituted in this rapid pacing model to examine the effects on LV function and dimensions in an evolving CHF process. The significant findings of the present study were 2-fold. First, concomitant MMP inhibition were improved in the rapid pacing and MMP inhibition group when compared with rapid pacing–only values.

LV Myocardial Collagen With Rapid Pacing: Effects of MMP Inhibition
LV myocardial fibrillar collagen structure and relative content were examined using picrosirius staining and histomorphometry (Figure 6). In the rapid pacing group, the fibrillar collagen weave surrounding individual myocytes appeared reduced and disrupted when compared with normal control sections. The relative collagen volume fraction was reduced in the rapid pacing group compared with control (4.8±0.8 versus 2.3±0.4%, respectively, P<0.05). In the rapid pacing and MMP inhibition group, the fibrillar collagen weave appeared increased between adjoining myocytes. In addition, specific regions of the myocardium contained a thickened fibrillar collagen weave. The overall LV myocardial volume were improved in the rapid pacing and MMP inhibition group when compared with rapid pacing–only values.

Figure 4. LV size and function were serially measured with each week of rapid pacing, with (n=7) and without (n=8) concomitant MMP inhibition (MMPi). Top, In the rapid pacing–only group, LV end-diastolic dimension increased in a time-dependent manner and was increased with each week of pacing (P<0.05). The degree of LV dilation was reduced in the MMP inhibition group when compared with rapid pacing–only values (P<0.05). Middle, After 1 week of rapid pacing, LV peak wall stress increased from baseline values in the untreated pigs (P<0.05) and increased from this value with longer durations of rapid pacing (P<0.05). At each week of pacing, LV peak wall stress was significantly lower in the rapid pacing and MMP inhibition group when compared with the rapid pacing–only group (P<0.05). Bottom, LV fractional shortening fell in a time-dependent manner in both the rapid pacing–only group and the MMP inhibition group. However, after 3 weeks of rapid pacing, LV fractional shortening was higher in the MMP inhibition group when compared with rapid pacing–only values. *P<0.05 vs rapid pacing–only values.
with rapid pacing reduced the degree of LV dilation and wall stress, which was accompanied by improved LV endocardial fractional shortening. Second, MMP inhibition during the progression of pacing CHF had minimal effects on indices of LV myocardial and myocyte contractility, which suggests that the basis for the improvement in LV fractional shortening was primarily a reduction in LV load. These findings suggest that a contributory mechanism for the LV remodeling that occurs during the progression of the CHF process is heightened MMP activity within the LV myocardium.

It has been reported previously that the institution of angiotensin-converting enzyme inhibition reduced the degree of LV dilation in a number of cardiac disease states. Furthermore, past studies have demonstrated that the effects of angiotensin-converting enzyme inhibition on LV geometry and function were likely due to local myocardial effects rather than to changes in systemic hemodynamics. Taken together, these past studies suggest that modulation of local neurohormonal and enzymatic pathways within the LV myocardium.

**TABLE 2. LV Geometry, LV Function, Systemic Hemodynamics, and Plasma Neurohormones With Chronic Rapid Pacing: Effects of Chronic MMPi**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Rapid Pacing*</th>
<th>Rapid Pacing†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>113±2</td>
<td>167±7‡</td>
<td>143±6§</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>93±1</td>
<td>80±3‡</td>
<td>81±2‡</td>
</tr>
<tr>
<td>LV size and function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End diastolic dimension, cm</td>
<td>3.55±0.05</td>
<td>5.67±0.11‡</td>
<td>4.92±0.17§</td>
</tr>
<tr>
<td>Wall thickness, cm</td>
<td>0.85±0.04</td>
<td>0.52±0.02‡</td>
<td>0.82±0.02§</td>
</tr>
<tr>
<td>Peak wall stress, g/cm²</td>
<td>136±3</td>
<td>360±13‡</td>
<td>187±11‡ §</td>
</tr>
<tr>
<td>Endocardial fractional shortening, %</td>
<td>45±1</td>
<td>19±2‡</td>
<td>25±2§</td>
</tr>
<tr>
<td>Midwall fractional shortening, %</td>
<td>21.5±0.4</td>
<td>14.6±1.6‡</td>
<td>15.8±0.9‡</td>
</tr>
<tr>
<td>Vcf, (s⁻¹)</td>
<td>2.8±0.2</td>
<td>1.6±0.3‡</td>
<td>2.0±0.3‡</td>
</tr>
<tr>
<td>Plasma neurohormones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renin activity, ng/mL per hour</td>
<td>3.1±0.6</td>
<td>16.2±4.2‡</td>
<td>11.2±1.2‡</td>
</tr>
<tr>
<td>Norepinephrine, pg/mL</td>
<td>210±35</td>
<td>1105±185‡</td>
<td>1085±211‡</td>
</tr>
<tr>
<td>Sample size, n</td>
<td>22</td>
<td>12</td>
<td>10</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
*Three weeks of supraventricular pacing at 240 bpm.
†Rapid pacing with concomitant treatment with the MMP inhibitor PD166793 (20 mg/kg QD).
‡P<0.05 vs baseline.
§P<0.05 vs rapid pacing only.
|Velocity of circumferential fiber shortening corrected for heart rate.

**Figure 5.** An LV ejection-stress relationship was determined for 5 control pigs through measurements of isochronal points during a phenylephrine infusion and were subjected to analysis to determine the LV endocardial fractional shortening–mean systolic wall stress relation (A) and the LV midwall shortening–mean systolic stress relation (B). Solid lines indicate the linear regression (fractional shortening-stress) relation for these isochronal points. Steady-state values obtained after 3 weeks of rapid pacing (●) or with rapid pacing and MMP inhibition (MMPi) treatment (◆) are shown. After chronic pacing, a significant downward and rightward shift was observed, which indicates a significant decline in LV ejection performance. With MMPi treatment during the pacing protocol, a reduction in LV wall stress was accompanied by improved LV pump function, which resulted in a left-upward shift in this relationship.
cardiomyocytes. 22–24 In light of the fact that MMP inhibition may potentially influence vascular wall architecture, future studies that are focused on measurements of vascular compliance with chronic MMP inhibition are warranted.

In the present study, concomitant MMP inhibition with rapid pacing was associated with an increase in LV endocardial fractional shortening when compared with untreated pacing CHF values. However, it must be recognized that the increase in this index of LV pump function was modest. The LV midwall shortening fraction has been used previously in the setting of LV hypertrophy to quantify myocardial fiber shortening in the circumferential orientation.42,43 In the present study, computed LV midwall shortening was significantly reduced in the pacing CHF group and was unchanged from these values with MMP inhibition. In both pacing CHF groups, the LV ejection-stress relation fell outside of normal limits, which indicated impaired LV myocardial contractile performance. Indeed, the present study demonstrated that the development of pacing-induced CHF was accompanied by diminished myocyte steady-state contractility, which was not significantly affected by MMP inhibition. Thus, with pacing CHF, both in vivo and in vitro indices of contractile performance were depressed and remained reduced with MMP inhibitor treatment. These results suggest that the basis for the increased LV endocardial fractional shortening with rapid pacing and MMP inhibition was primarily a favorable effect on LV remodeling rather than an intrinsic effect on LV myocardial contractile performance.

An important determinant of collagen degradation is activation of the MMPs, which have high selectivity and affinity for components of the extracellular matrix.18–24 MMPs are secreted in a proenzyme form and are activated through a number of enzymatic pathways.18,19,21 Several past studies using in vitro methodologies have demonstrated that LV myocardial MMP activity was increased in association with changes in LV geometry.5,6,25–27,59 Increased LV myocardial MMP activity has been demonstrated in human end-stage cardiomyopathic disease.26,27 Consistent with these past reports, the present study demonstrated significantly increased MMP zymographic activity in LV myocardial extracts after the development of pacing-induced CHF. However, this in vitro observation does not address whether in vivo MMP activity directly contributed to the LV remodeling process with developing CHF. The present study demonstrated that in vivo MMP inhibition significantly influenced the LV remodeling process with chronic rapid pacing. Results from the present study demonstrated that LV MMP zymographic activity, an index of LV myocardial MMP abundance, was unchanged from pacing CHF values with concomitant MMP inhibition. These observations suggest that the attenuation of LV dilation that was achieved with MMP inhibition did not cause a reduction in overall LV myocardial MMP content. Past studies from this laboratory have demonstrated that pacing CHF is associated with increased expression of a number of species of MMPs within the myocardium.5,6 On the basis of the results from the present study, future studies that use more quantitative measures of myocardial MMP

### TABLE 3. Isolated Myocyte Contractile Function With Pacing-Induced Congestive Heart Failure: Effects of Chronic MMPi

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>25 mmol/L Isoproterenol</th>
<th>8 mmol/L Calcium</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Resting length, μm</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>125±4</td>
<td>120±4</td>
<td>118±5</td>
</tr>
<tr>
<td>Rapid pacing*</td>
<td>180±3†</td>
<td>169±2††</td>
<td>165±7††</td>
</tr>
<tr>
<td>Rapid pacing + MMPi§</td>
<td>169±4††</td>
<td>161±3††</td>
<td>156±5††</td>
</tr>
<tr>
<td><strong>Percentage of shortening, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.8±0.2</td>
<td>11.7±0.9‡</td>
<td>9.5±0.4‡</td>
</tr>
<tr>
<td>Rapid pacing*</td>
<td>2.3±0.1†</td>
<td>4.7±0.3‡‡</td>
<td>4.9±0.8‡‡</td>
</tr>
<tr>
<td>Rapid pacing + MMPi§</td>
<td>2.2±0.1†</td>
<td>5.1±0.5‡‡</td>
<td>5.6±0.5‡‡</td>
</tr>
<tr>
<td><strong>Shortening velocity, μm/s</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>66±1</td>
<td>204±17††</td>
<td>119±9‡</td>
</tr>
<tr>
<td>Rapid pacing*</td>
<td>33±2‡</td>
<td>94±9†</td>
<td>69±10†</td>
</tr>
<tr>
<td>Rapid pacing + MMPi§</td>
<td>31±2†</td>
<td>108±11††</td>
<td>84±7‡†</td>
</tr>
<tr>
<td><strong>Relengthening velocity, μm/s</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>67±4</td>
<td>169±15§</td>
<td>124±11‡</td>
</tr>
<tr>
<td>Rapid pacing*</td>
<td>29±2†</td>
<td>62±5‡†</td>
<td>64±9‡†</td>
</tr>
<tr>
<td>Rapid pacing + MMPi§</td>
<td>26±2†</td>
<td>80±11‡†</td>
<td>78±5‡†</td>
</tr>
<tr>
<td><strong>Time to peak contraction, ms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>235±10</td>
<td>183±4‡</td>
<td>262±13</td>
</tr>
<tr>
<td>Rapid pacing*</td>
<td>267±8†</td>
<td>198±4‡‡</td>
<td>274±10</td>
</tr>
<tr>
<td>Rapid pacing + MMPi§</td>
<td>244±7‖</td>
<td>175±2‖</td>
<td>240±3‖</td>
</tr>
<tr>
<td><strong>Time to 50% relaxation, ms</strong></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>90±4</td>
<td>71±5‖</td>
<td>86±7</td>
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<tr>
<td>Rapid pacing*</td>
<td>134±5†</td>
<td>124±5†</td>
<td>127±14†</td>
</tr>
<tr>
<td>Rapid pacing + MMPi§</td>
<td>131±5†</td>
<td>104±6‖</td>
<td>102±5‖</td>
</tr>
<tr>
<td><strong>Total duration, ms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>501±17</td>
<td>380±15†</td>
<td>472±18</td>
</tr>
<tr>
<td>Rapid pacing*</td>
<td>533±13</td>
<td>488±15‖</td>
<td>556±28†</td>
</tr>
<tr>
<td>Rapid pacing + MMPi§</td>
<td>498±9‖</td>
<td>433±12‖</td>
<td>476±10‖</td>
</tr>
</tbody>
</table>

Values are presented as mean±SEM.  
*Three weeks of supraventricular pacing at 240 bpm.  
†P<0.05 vs control.  
‡P<0.05 vs baseline.  
‖P<0.05 vs rapid pacing.
species expression with chronic MMP inhibition are warranted. Nevertheless, the unique results from the present study provide direct evidence to suggest that a contributory mechanism for the LV myocardial remodeling that occurs during the progression of a CHF process is increased LV myocardial MMP activity.

Consistent with past reports, the LV dilation that occurred with chronic rapid pacing was not associated with a concomitant increase in LV mass. Thus, significant LV myocardial remodeling must occur in this model of pacing-induced CHF. The development of pacing-induced CHF was associated with increased myocyte length. The increased myocyte length parallels the significant LV dilation and myocardial remodeling that occurs in this CHF process.

In the present study, MMP inhibition during chronic rapid pacing reduced resting myocyte length. These findings suggest that a contributory cellular mechanism for the reduction in LV dilation with MMP inhibition during chronic rapid pacing is a reduction in myocyte length. As demonstrated in the present study, the increased LV dimension and reduced wall thickness with pacing-induced CHF resulted in significantly elevated LV peak wall stress. The relative degree of LV dilation and wall thinning with concomitant MMP inhibition and rapid pacing was attenuated. Past results provide evidence to suggest that mechanisms for the failure of a significant hypertrophic response with pacing-induced CHF include reduced myocyte cross-sectional area, changes in cytoskeletal protein expression, increased contractile protein degradation rates, and induction of myocyte cell death through apoptosis. Whether and to what degree MMP inhibition influenced these cellular and molecular processes with pacing CHF remains to be established.

In light of the fact that the development of CHF is associated with significant alterations in LV loading conditions and neurohormonal systems, the determination of intrinsic properties of contractile performance in vivo can be difficult. Accordingly, isolated LV myocyte contractile function was measured in the normal control state, after chronic rapid pacing, and after MMP inhibition with chronic pacing. Consistent with past reports, the development of pacing CHF resulted in diminished indices of steady-state contractile function. Furthermore, pacing-induced CHF caused diminished myocyte inotropic responsiveness after β-adrenergic receptor stimulation or in the presence of increased extracellular Ca^2+. MMP inhibition during chronic rapid pacing did not significantly improve steady-state contractile function and had minimal effects on myocyte inotropic response. It has been demonstrated previously that the rate of LV isovolumic pressure decline, which is an index of active myocardial relaxation, is altered with pacing CHF. Indices of active myocyte relaxation, such as the velocity of myocyte lengthening and time to 50% relaxation, were prolonged with pacing CHF. These defects in active myocyte relaxation likely contribute to the prolonged time of isovolumic relaxation that has been reported previously. Certain indices of myocyte active relaxation, such as time to 50% relaxation, were reduced in the MMP inhibition group when compared with untreated CHF values. Whether this decrease in the time to 50% of myocyte relaxation can be translated into a measurable change in LV isovolumic relaxation under ambient in vivo conditions remains to be established. It has been postulated that an important system for the translation of myocyte shortening into an overall LV ejection is the collagen-integrin-myocyte cytoskeletal complex. Abnormalities in LV myocyte interactions with the extracellular domain have been reported to occur with pacing CHF. Thus, MMP inhibition may have resulted in improved transduction of myocyte shortening into an LV ejection.

The reduced LV dilation that was observed with concomitant MMP inhibition during rapid pacing may have been due, at least in part, to increased myocardial collagen content and improved extracellular support. MMP inhibition was associated with a relative increase in collagen content from both untreated CHF and control values. Changes in the relative composition of the myocardial fibrillar collagen matrix have been implicated in the influence of LV myocardial stiffness characteristics. Consistent with past reports from a number of laboratories, the present study demonstrated that the LV dilation and myocardial remodeling that occurs with pacing CHF is not associated with significant alterations in LV chamber or myocardial stiffness properties. In an isolated LV heart preparation, Wolff et al reported a...
significant overlap between the diastolic stress-strain relation in normal and pacing CHF preparations. In the present study, indices of LV chamber and myocardial stiffness increased with MMP inhibition. A number of factors influence LV chamber stiffness, such as loading conditions and myocardial active relaxation processes, as well as myocardial stiffness. The findings from the present study demonstrated that the increased LV chamber stiffness with MMP inhibition was likely due to an absolute increase in LV myocardial stiffness. Given that LV myocardial stiffness reflects intrinsic material properties of the myocardium itself, this increase in myocardial stiffness with MMP inhibition was likely due to changes in myocardial collagen content and structure. Thus, whereas MMP inhibition reduced LV chamber dimensions during the development of pacing CHF, this was accompanied by negative effects on LV chamber compliance characteristics and myocardial stiffness properties.

It must be recognized that the present study used only one dosing regimen of MMP inhibition. This dose, which was selected on pharmacokinetic studies, was demonstrated to induce physiological and biological responses in this pacing model of CHF with respect to LV dimensions and wall stress patterns, LV compliance properties, myocardial MMP activity, and relative collagen content. Using an ex vivo gelatinolytic assay system that provided an index of MMP activity in crude LV myocardial extracts, an attenuation of MMP activity after APMA stimulation was significantly reduced from basal values in the rapid pacing (Pace) samples. In the rapid pacing and MMP inhibition (Pace/MMPi) group, LV myocardial gelatinase activity was significantly lower after APMA treatment when compared with pacing CHF values. *P<0.05 vs basal values; †P<0.05 vs pacing CHF.

In the present study, concomitant MMP inhibition ameliorated the LV dilation and increased wall stress that invariably occurs with the progression of pacing CHF. However, it must be recognized that chronic MMP inhibition did not completely prevent the changes in LV size with chronic rapid pacing. In the current experimental preparation, LV stroke volume and cardiac output were not directly measured. It has been demonstrated previously that this model of pacing CHF is associated with an exhaustion of the Frank-Starling mechanism. Thus, whether dose-dependent effects of MMP inhibition exist at the level of the myocardium or to what degree alternative dosing strategies would influence the degree of LV dilation, collagen accumulation and myocardial stiffness properties warrant further study.

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There are a number of species of MMPs that have different specificities to the fibrillar collagens. Moreover, an important control point of MMP activity is the tissue inhibitors of MMPs (TIMPs). These TIMPs form tight complexes with MMPs and therefore play an important role.
in overall MMP enzymatic activity. The MMP assays performed in the present study could not address whether potential changes in TIMP abundance and/or the stoichiometric relation to specific MMPs may have occurred with pacing CHF or with concomitant MMP inhibition. Thus, an important future direction will be to examine how the activity of specific species of MMPs and the relative association with TIMPs influence the LV remodeling process in the setting of developing CHF. It has been demonstrated previously that a number of proteolytic enzyme systems exist that can contribute to the tissue remodeling process.32,33 For example, the serine proteinases such as plasmin, kallikrein, and elastase make up the largest class of mammalian proteinases and contribute to extracellular remodeling.23 In the present study, MMP inhibition did not completely prevent the degree of LV dilation with rapid pacing. Thus, it is likely that other proteolytic systems contribute to the LV remodeling process in this model of CHF. Nevertheless, the unique findings of the present study suggest that heightened LV myocardial MMP activity contributes to LV remodeling during the progression of the CHF process.

Acknowledgments

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Matrix Metalloproteinase Inhibition During the Development of Congestive Heart Failure: Effects on Left Ventricular Dimensions and Function


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