

# Angiotensin-Converting Enzyme and Matrix Metalloproteinase Inhibition with Developing Heart Failure: Comparative Effects on Left Ventricular Function and Geometry<sup>1</sup>

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## ABSTRACT

The progression of congestive heart failure (CHF) is left ventricular (LV) myocardial remodeling. The matrix metalloproteinases (MMPs) contribute to tissue remodeling and therefore MMP inhibition may serve as a useful therapeutic target in CHF. Angiotensin converting enzyme (ACE) inhibition favorably affects LV myocardial remodeling in CHF. This study examined the effects of specific MMP inhibition, ACE inhibition, and combined treatment on LV systolic and diastolic function in a model of CHF. Pigs were randomly assigned to five groups: 1) rapid atrial pacing (240 beats/min) for 3 weeks ( $n = 8$ ); 2) ACE inhibition (fosinopril, 2.5 mg/kg b.i.d. orally) and rapid pacing ( $n = 8$ ); 3) MMP inhibition (PD166793 2 mg/kg/day p.o.) and rapid pacing ( $n = 8$ ); 4) combined ACE and MMP inhibition (2.5 mg/kg b.i.d. and 2 mg/kg/day, respectively) and rapid pacing

( $n = 8$ ); and 5) controls ( $n = 9$ ). LV peak wall stress increased by 2-fold with rapid pacing and was reduced in all treatment groups. LV fractional shortening fell by nearly 2-fold with rapid pacing and increased in all treatment groups. The circumferential fiber shortening-systolic stress relation was reduced with rapid pacing and increased in the ACE inhibition and combination groups. LV myocardial stiffness constant was unchanged in the rapid pacing group, increased nearly 2-fold in the MMP inhibition group, and was normalized in the ACE inhibition and combination treatment groups. Increased MMP activation contributes to the LV dilation and increased wall stress with pacing CHF and a contributory downstream mechanism of ACE inhibition is an effect on MMP activity.

A milestone in the development and progression of congestive heart failure (CHF) is alterations in left ventricular (LV) geometry, commonly referred to as myocardial remodeling. LV myocardial remodeling and subsequent chamber dilation have been associated with increased morbidity and mortality in patients with CHF. Angiotensin-converting enzyme (ACE) inhibition with developing CHF improves survival in patients with CHF, and a postulated mechanism for this beneficial effect is an attenuation of the LV remodeling process (Konstam et al., 1993; Greenberg et al., 1995). Thus, LV

remodeling is probably an important structural contributory event in the progression to end-stage CHF. However, the cellular and molecular bases for the changes in LV geometry that occur during the progression of CHF remain poorly understood. An important constituent of the LV myocardium is the fibrillar collagen matrix, which has been proposed to contribute to the maintenance of LV geometry and the structural alignment of adjoining myocytes (Borg et al., 1990; Weber et al., 1992). 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 (Weber et al., 1988b; Spinale et al., 1991a, 1995; Komamura et al., 1993; Gunja-Smith et al., 1996). An important consequence with respect to LV remodeling in CHF is alterations in LV wall stress patterns. Fundamental determinants of LV peak and systolic wall stress are chamber dimension and myocardial wall thickness. The progressive LV dilation and wall remodeling that occurs with CHF will result in increased LV wall stress, which in turn

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**ABBREVIATIONS:** CHF, congestive heart failure; LV, left ventricular; ACE, angiotensin-converting enzyme; MMP, matrix metalloproteinase; Ang-I, angiotensin I; PRSWR, preload recruitable stroke work relation; Vcfc, velocity of circumferential fiber shortening;  $K_c$ , chamber stiffness constant;  $K_m$ , myocardial stiffness constant; TIMP, tissue inhibitor of matrix metalloproteinase; AP-1, activator protein 1.

will be translated into a higher LV afterload to the compromised LV myocardium. Thus, the LV remodeling with CHF can directly contribute to the progression and/or exacerbation of the LV pump dysfunction that occurs in this disease state. These observations would support the concept that modulation of the LV myocardial remodeling process would be an important therapeutic strategy in the setting of developing CHF.

An endogenous family of enzymes responsible for extracellular collagen degradation and remodeling is the matrix metalloproteinases (MMPs) (Hansen-Berkedal et al., 1993; Rees et al., 1993; Dollery et al., 1995). This laboratory and others have recently identified increased expression and activity of MMPs within the LV myocardium in both patients and animals with CHF (Gunja-Smith et al., 1996; Spinale et al., 1998; Thomas, 1998). The recent development of bioavailable MMP inhibitors makes it possible to modulate MMP activity *in vivo* and to assess the effects of MMP inhibition on the tissue remodeling process (Hodgson, 1995; Taraboletti et al., 1995; Watson et al., 1996). Accordingly, the first goal of this study was to identify the effects of MMP inhibition on indices of LV geometry and function with developing CHF. In light of the fact that ACE inhibition influences LV geometry and function with CHF, the second goal of this study was to quantitatively determine the potential synergistic/additive effects of combined ACE and MMP inhibition on LV geometry and function with CHF. Chronic rapid pacing in animals causes a spectrum of changes in LV functional and neurohormonal profiles that resemble the clinical phenotype of dilated cardiomyopathy (Spinale, 1991a; Spinale et al., 1991b, 1995, 1997, 1998; Tomita et al., 1991; Komamura et al., 1993). This model of pacing induced CHF causes progressive LV dilation and myocardial remodeling, which is accompanied by both systolic and diastolic dysfunction (Tomita et al., 1991; Komamura, 1993). More recently, it has been demonstrated that MMP activity was increased early in the progression of pacing CHF and was temporally related to the onset of the LV dilation and pump dysfunction (Spinale et al., 1998). Accordingly, this model of CHF was used in the present study to examine the effects of MMP inhibition, ACE inhibition, or combination treatment on indices of LV systolic and diastolic performance.

## Materials and Methods

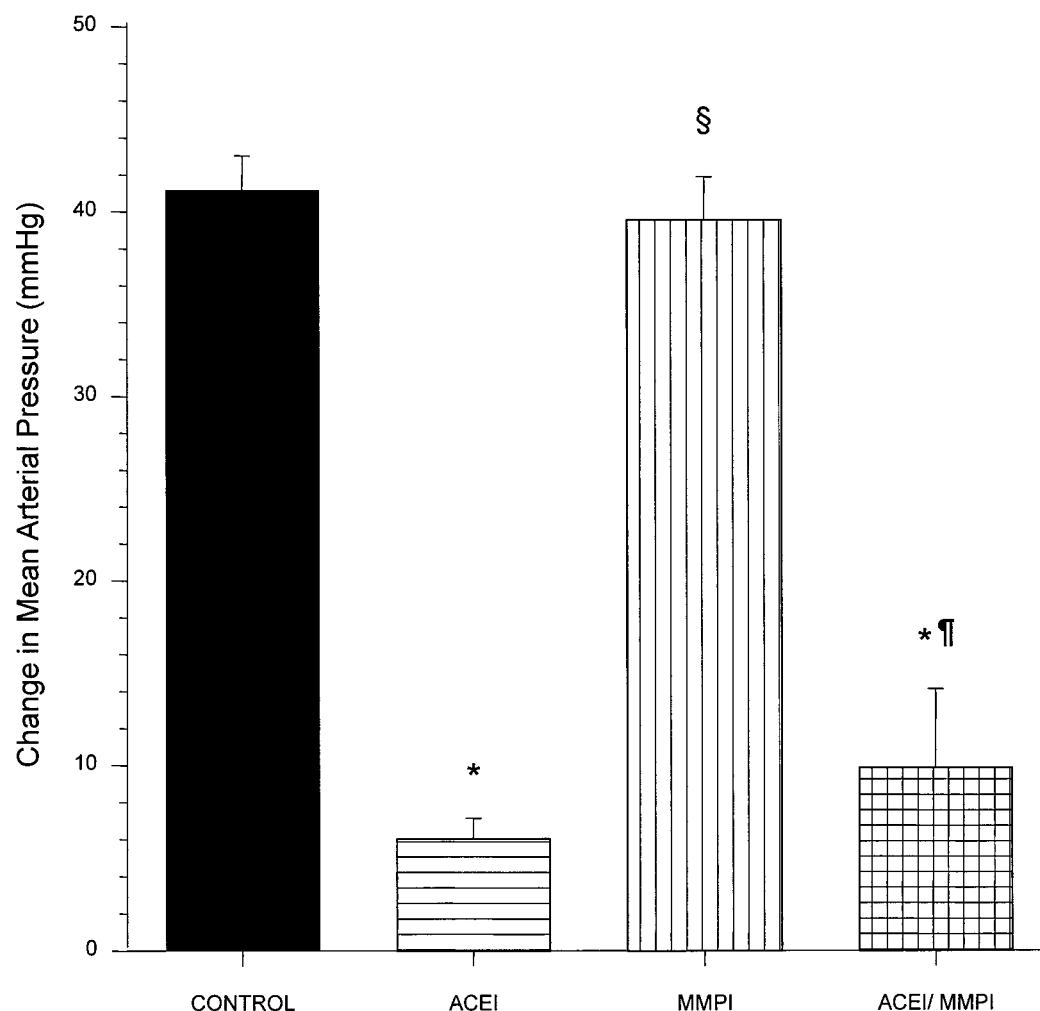
**Rationale.** The first objective of this study was to select an appropriate dosing strategy for MMP inhibition. A large number of MMP species exists that possess differential substrate specificities (Hansen-Birkedal et al., 1993; Rees et al., 1993; Werb and Alexander, 1993; Dollery et al., 1995; Nagase, 1997). To examine the generalized effects of MMP inhibition on LV remodeling, the present study used an MMP inhibitor that would provide global plasma MMP inhibitory activity. The second objective was to establish the dosing strategy for ACE inhibition and combined MMP inhibition. For these studies, the criterion for adequate ACE inhibition was to effectively inhibit the pressor response to an angiotensin I (Ang-I) infusion without producing hemodynamic instability (Spinale et al., 1995, 1997). After identification of the dosing regimen, treatment with MMP inhibition, ACE inhibition, or combined inhibition was initiated in chronically instrumented pigs undergoing rapid pacing. LV size and function were measured with each week, and after 21 days of concomitant treatment and rapid pacing, terminal studies were performed in which LV systolic and diastolic function were examined. For

comparison purposes, age-matched pigs that underwent chronic pacing without treatment as well as sham controls were used.

**Dose Selection Studies.** Ten Yorkshire pigs (20 kg, male; Orangeburg, Hambone Farms, SC) were chronically instrumented to measure aortic blood pressure in the conscious state as described previously (Spinale et al., 1997, 1998). Briefly, under isoflurane anesthesia (3% in 1.5 l/min oxygen) and through a left thoracotomy, a catheter connected to a vascular access port (model GPV, 9F; Access Technologies, Skokie, IL) was placed in the thoracic aorta and sutured in place. The access port was buried in a s.c. pocket over the thoracolumbar fascia. After a recovery period of 7 to 10 days, the animal was returned to the laboratory for an initial Ang-I pressor response study. For these studies, the animals were sedated with diazepam (20 mg p.o. valium; Hoffmann-La Roche Inc., Nutley, NJ) and placed in a custom-designed sling that allowed the animal to rest comfortably. The vascular access port was entered with a 20-gauge Huber needle (Access Technologies) and resting aortic pressure and heart rate were recorded. Pressures from the fluid-filled aortic catheter were obtained with an externally calibrated transducer (Statham P23ID; Gould Inc., Oxnard, CA). The ECG and pressure waveforms were recorded and digitized to computer for subsequent analysis at a sampling frequency of 100 Hz (NI-DAQ; National Instruments, Austin, TX). Following these baseline measurements, Ang-I (10  $\mu$ g; Sigma Chemical Company, St. Louis, MO) was administered through the access port and measurements recorded 10 min after infusion.

Following the control studies, five pigs were administered the ACE inhibitor fosinopril (Bristol-Myers-Squibb, Princeton, NJ) at a dose of 2.5 mg/kg b.i.d. for 3 days and the Ang-I studies repeated. This dose of fosinopril was selected on preliminary dose-ranging studies. Resting mean aortic pressure was reduced with ACE inhibition compared with controls ( $79 \pm 4$  versus  $96 \pm 2$  mm Hg,  $p < .05$ ) and significantly blunted the Ang-I pressor response (Fig. 1). The MMP inhibitor chosen for this study was PD166793 (Parke-Davis, Ann Arbor, MI), which has global MMP inhibitory activity based on both *in vitro* and *in vivo* assay systems (Parke-Davis, T.J.P., personal communication). Five pigs underwent 2 mg/kg every day in the morning of treatment with PD166793 for 3 days. This dosing protocol was selected based on initial pharmacokinetic studies. At 24 h after the third day of treatment, trough plasma PD166793 levels were  $8.25 \pm 0.35$   $\mu$ g/ml. This plasma level of PD166793 resulted in >50% reduction in MMP inhibitory activity based on an *ex vivo* assay system. Briefly, this *ex vivo* assay system was based on the degree of hydrolysis of the MMP substrate thiopeptolide Ac-Pro-Leu-Gly-thioester-Leu-Leu-Gly-Oet, which has been described previously (Ye et al., 1992, 1994, 1995). Although this dosing strategy provided significant plasma MMP inhibitory activity, there were no effects on resting hemodynamics or the Ang-I pressor response compared with control values (Fig. 1). Finally, five pigs were treated with combined ACE (2.5 mg/kg b.i.d.) and MMP (2 mg/kg every day in the morning) inhibition for 3 days and steady-state blood pressure and Ang-I pressor response were examined. Resting mean aortic pressure was reduced from control values ( $68 \pm 5$  mm Hg,  $p < .05$ ) but resting heart rate was unchanged. The Ang-I pressor response was significantly blunted in the combined treatment protocol but was similar to ACE-inhibition-only values (Fig. 1).

**Experimental Protocol and Animal Model Preparation.** After the dose selection studies, the effects of concomitant treatment with MMP inhibition, ACE inhibition, or combined inhibition with chronic rapid pacing were examined. Weight-matched pigs (22–23 kg) were randomly assigned to five groups: 1) rapid atrial pacing (240 beats/min) for 3 weeks ( $n = 8$ ); 2) concomitant ACE inhibition (fosinopril, 2.5 mg/kg b.i.d. orally) and rapid pacing ( $n = 8$ ); 3) concomitant MMP inhibition (PD166793 2 mg/kg/day p.o.) and rapid pacing ( $n = 8$ ); 4) combined ACE and MMP inhibition (2.5 mg/kg b.i.d. and 2 mg/kg/day, respectively) and rapid pacing ( $n = 8$ ); and 5) sham controls, instrumentation with no rapid pacing ( $n = 9$ ). All drug



**Fig. 1.** The percentage of change in mean aortic pressure following infusion of 10  $\mu$ g of Ang-I in conscious pigs. ACE inhibition (ACEI; fosinopril, 2.5 mg/kg b.i.d.) for 3 days caused a significant reduction in the Ang-I pressor response. MMP inhibition (MMPI; PD166793, 2 mg/kg/day p.o.) for 3 days did not change the Ang-I pressor response from control values. Combined ACE and MMP inhibition (ACEI/MMPI; 2.5 mg/kg b.i.d. and 2 mg/kg/day, respectively) for 3 days reduced the Ang-I pressor response from control values but was similar to ACE-inhibition-only values (\* $p$  < .05 versus untreated control; § $p$  < .05 versus ACEI; ¶ $p$  < .05 versus MMP inhibition).

treatments were started 3 days before the initiation of pacing and continued for the entire 21-day pacing protocol.

For these studies, the animals were anesthetized as described in the preceding section and the aortic access port was placed. In addition, a shielded stimulating electrode was sutured onto the left atrium, connected to a modified programmable pacemaker (8329; Medtronic Inc., Minneapolis, MN) and buried in a s.c. pocket. Ten to 14 days after recovery from the surgical procedure, baseline studies were performed and the protocols described above were begun. Cardiac auscultation and an ECG were performed frequently during the pacing protocol to ensure proper operation of the pacemaker and the presence of 1:1 conduction. In this porcine preparation, atrioventricular conduction can be maintained at this pacing rate and therefore can provide a homogeneous pattern of ventricular myocardial electrical activation. The sham-operated controls were cared for in identical fashion with the exception of the pacing protocol. All animals were treated and cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**LV Function and Hemodynamic Measurements.** LV size and function were measured at weekly intervals in all of the pigs entered in the protocol. All of these studies were performed 2 to 3 h after the morning drug treatments. 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 min following pacemaker deactivation. Aortic line access was established and resting, ambient heart rate and pressures were recorded. Two-dimensional and M-mode echocardiographic studies (ATL Ultramark VI, 2.25-MHz transducer; Advanced Technical Laboratories, Bothell, WA) were used to image the LV

from a right parasternal approach. LV fractional shortening was calculated as (end-diastolic dimension – end-systolic dimension)/end-diastolic dimension and was expressed as a percentage. Peak circumferential global average wall stress was computed with a spherical model of reference:  $\sigma(\text{g/cm}^2) = (\text{PD}/4h(1 + h/D)) \times 1.36$ , where P = aortic systolic pressure measured from the access port, D = minor axis dimension at end-diastole, and h = wall thickness at end-diastole. At day 21 of the study protocol, a final set of LV function measurements was performed and blood was collected for neurohormonal assay and drug level measurements. The blood samples were immediately centrifuged (2000g, 10 min, 4°C), the plasma decanted into separate tubes, and stored at –80°C until the time of assay.

**LV Ejection Performance and Hemodynamics.** After the final set of LV echocardiographic studies and plasma collection, the pigs were anesthetized for a more comprehensive study of LV function and hemodynamics. All of these studies were performed 6 to 8 h after the morning drug treatment. A bolus of 1  $\mu$ g/kg sufentanyl was administered, an endotracheal tube placed, and mechanical ventilation initiated. Anesthesia was maintained throughout the procedure by delivery 0.5  $\mu$ g/kg/h sufentanyl. In our preliminary studies, this anesthetic protocol resulted in a deep anesthetic plane and stable hemodynamic profiles for up to 6 h. After intubation, pancuronium (0.1 mg/kg) was administered and this dose repeated every 45 min during the procedure. A multilumenated thermodilution catheter (7.5F; Baxter Healthcare Corp., Irvine, CA) was positioned in the pulmonary artery via the right external jugular vein. A sternotomy was performed and a vascular ligature was placed around the inferior vena cava to perform transient caval occlusion. A previously



calibrated microtipped transducer (7.5 F; Millar Instruments, Inc., Houston, TX) was placed in the LV through a small apical stab wound. Four piezoelectric crystals (2 mm; Sonometrics, Ontario, Canada) were positioned on the LV anterior free wall to obtain orthogonal myocardial dimensions. One crystal was placed on the LV endocardial surface through a small myocardial incision and sutured in place. The three remaining crystals were placed at equidistantly on the LV epicardial surface to form a triangular array around the endocardial crystal. From this crystal array, six unique distances between crystal pairs were recorded at a sampling frequency of 100 Hz and digitized (Pentium-Sonolab; Sonometrics). After the placement of the instrumentation, baseline hemodynamics were recorded and digitized. Thermodilution-derived cardiac output and stroke volume were obtained from the pulmonary artery catheter in triplicate. All measurements were simultaneously recorded with the ventilator temporarily suspended to prevent respiratory artifact in the LV pressure recordings. Following steady-state measurements, LV preload was altered by sequential occlusion and release of the inferior vena cava and measurements recorded during occlusion and release. This maneuver was repeated in triplicate and used to derive indices of LV systolic and diastolic performance as described in the following paragraph.

After the final set of measurements, the animals were deeply anesthetized (5% isoflurane), and the heart was quickly extirpated and placed in a phosphate-buffered ice slush. The region of the LV free wall incorporating the circumflex artery (5 × 5 cm) was excised and perfused with 4% buffered formaldehyde (Formalin) at 50 mm Hg perfusion pressure and then processed for morphometric measurements. The region of the LV free wall comprising the left anterior descending artery (3 × 5 cm) was cannulated and perfused with a buffered sodium cacodylate solution containing 2% paraformaldehyde and 2% glutaraldehyde solution (pH 7.4, 325 mOsm) for 20 min with a perfusion pressure of 50 mm Hg and prepared for scanning electron microscopy. The LV posterior wall (3 × 3 cm) was quickly cut into small cubes and processed for MMP zymographic activity.

**Computations.** LV peak positive  $dp/dt$  was determined from the digitized LV pressure signal. Pulmonary and systemic vascular resistances were computed from the thermodilution cardiac output and pressure measurements with standard formulae (Spinale et al., 1997). From the digitized piezoelectric crystal data, LV myocardial wall thickness throughout the cardiac cycle was computed through triangulation of the six distance pairs with a Cartesian frame of reference. LV end-diastolic myocardial wall thickness was considered to be the minimum wall thickness measurement obtained during the cardiac cycle and was temporally aligned with the R-wave of the simultaneously digitized ECG. LV end-systolic wall thickness was defined as maximum myocardial wall thickness. LV mass was calculated with the LV chamber dimensions and sonometrically determined myocardial wall thickness by previously described and validated formulae (Spinale et al., 1998). Because it can be assumed that LV mass does not change throughout the cardiac cycle, then LV volumes could be derived throughout the cardiac cycle with the myocardial crystal measurements. From these computations, the isochronal LV end-diastolic pressure-stroke work points following release of the caval occlusion were then subjected to linear regression methods (Colan et al., 1984; McClain et al., 1998). The slope of this relationship has been defined as the LV preload recruitable stroke work relation (PRSWR) (Colan et al., 1984; McClain et al., 1998). LV myocardial velocity of circumferential fiber shortening, corrected for heart rate ( $V_{cf}$ ) was computed from the digitized LV crystal and pressure data as described previously (McClain et al., 1998). The isochronal LV  $V_{cf}$ -end-systolic wall stress relation also was determined following release of the caval occlusion.

Indices of LV diastolic function were determined by computations of the regional LV chamber stiffness constant ( $K_c$ ) and myocardial stiffness constant ( $K_m$ ) (Spinale et al., 1991a; Tomita et al., 1991). Calculations for  $K_c$  were based on analyses of the LV end-diastolic pressure versus LV end-diastolic chamber dimension (Tomita et al.,

1991). Briefly, the relationship between LV end-diastolic pressure and chamber dimension were fitted to an exponential function given by  $P = Ae^{K_c D}$  where  $P$  is LV end-diastolic pressure,  $D$  is chamber dimension, and  $A$  and  $K_c$  were fitting constants. The natural logarithm of this function was used to compute  $K_c$  by linear regression. Calculations of  $K_m$  were based on the analyses of the stress-strain relationship (Tomita et al., 1991). Isochronal LV end diastolic stress and natural strain were computed with release of the caval occlusion. LV end-diastolic wall stress was computed with a spherical frame of reference as described in the previous section and the natural strain was computed as  $\ln(D/D_0)$ , where  $D_0$  was defined as the LV end diastolic dimension at the lowest end-diastolic pressure immediately following the release of caval occlusion. The exponential stress-strain relationship was linearized by a log transformation and  $K_m$  defined as the slope derived from the regression analysis.

**Neurohormonal Measurements.** The plasma samples were assayed for renin activity, catecholamine levels, and plasma MMP inhibitor levels. Plasma renin activity was determined by computing Ang-I production with a radioimmunoassay (NEA-026; New England Nuclear, Boston, MA). Plasma norepinephrine was measured with HPLC and normalized to picograms per milliliter of plasma. Plasma concentrations of the MMP inhibitor were determined by HPLC and mass spectroscopy. All assays were performed in duplicate.

**LV Morphometric Analysis.** Light microscopic examination was performed on the perfusion fixed LV myocardium to determine the percentage of area occupied by fibrillar collagen (Spinale et al., 1991a, 1998). LV sections were stained with a picro-sirius technique (Spinale et al., 1998). The stained LV sections were imaged under polarized light and digitized at a final magnification of 320×. The images were obtained from 15 random fields within the midmyocardium to exclude large epicardial arteries and veins and any cutting or compression artifact. The fluorescent signal was then quantitated (Image-Pro Plus; Media Cybernetics, Silver Spring, MD) and mean collagen volume fraction calculated by averaging the sum of all collagen areas and dividing it by the sum of all extracellular and muscle area and expressing it as a percentage. The LV sections for scanning electron microscopic analysis were perfused with a buffered sodium cacodylate solution containing 2% paraformaldehyde, 2% glutaraldehyde solution (pH 7.4, 325 mOsm) for 20 min with a perfusion pressure of 100 mm Hg. Following perfusion, a 2 × 2-cm region was placed in additional fixative for 3 h, flash frozen in liquid nitrogen, and freeze fractured (Spinale et al., 1991). The freeze-fractured samples (0.25 × 0.25 cm) were then dehydrated and critical point dried (Ladd Research Inc., Burlington, VT). The samples were mounted on 10 × 10-mm stubs with conductive adhesive tape (Scotch commercial tape; 3M Inc., St. Paul, MN), and gold sputter-coated (Hummer II, Technics, Springfield, VA). The sections were examined in a JOEL JSM-25S scanning electron microscope at an accelerating voltage of 15kV.

**LV Myocardial Zymographic Activity.** Relative LV MMP activity was examined by substrate-specific zymographic analysis as described previously (Spinale et al., 1998; Thomas et al., 1998). The LV myocardial samples were extracted in an MMP stabilization buffer that prevented autolytic activity. The stepwise activation of MMPs can be elicited by serine proteases such as trypsin or plasmin (Nagase, 1997). Accordingly, LV myocardial extracts were incubated with trypsin (0.5 µg/ml, type I; EC 3.4.21.4, 5 min at 37°C) to unfold the MMP enzyme and cleave the activation peptide sequence for maximal MMP zymographic activity (Thomas et al., 1998). The LV myocardial extracts were loaded onto electrophoretic gels (SDS-polyacrylamide gel electrophoresis) containing 1 mg/ml gelatin. Following SDS-polyacrylamide gel electrophoresis, the gels were washed and incubated for 12 h in a MMP substrate buffer at 37°C. Following incubation, the gels were stained with 0.1% Amido Black and destained in water. The zymograms were digitized and the size-fractionated banding pattern, which indicated MMP proteolytic activity, was determined by quantitated image analysis (Gel Pro Analyzer; Media Cybernetics, Silver Spring, MD). The lysis areas were

measured by two-dimensional integrated optical density computations and expressed in pixels. In an additional series of studies with normal LV myocardial extract preparations, MMP zymographic activity was examined in the presence of 1 to 50  $\mu\text{g/ml}$  PD166793. In these studies, the MMP inhibitor was included in the MMP substrate buffer and all experiments were repeated in triplicate.

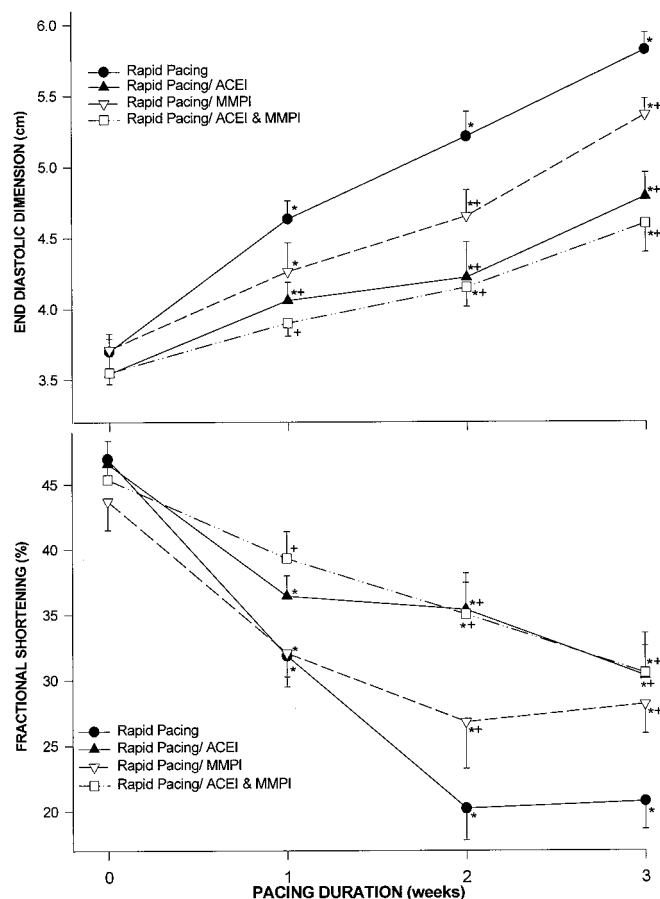
**LV Myocardial ACE Activity.** Myocardial tissue ACE activity was measured with a modified procedure described previously (Spinale et al., 1997). Briefly, LV myocardial samples were homogenized in phosphate buffer (50 mM, pH 7.5) containing Triton X-100 (0.3%) and the insoluble protein was removed through centrifugation (1500 rpm, 20 min). The samples ( $\sim 50 \mu\text{g}$  of total protein) were incubated 1:3 with sodium phosphate buffer (50 mM, pH 7.5) containing hippuryl-His-Leu for 10 min at  $37^\circ\text{C}$ . The formation of His-Leu, which reflects ACE activity, was then stopped with the addition of excess NaOH, and the reaction mixture was labeled with 2% o-phthalaldehyde. The labeled product formed was then quantified fluorometrically (Turner-112 Fluorimeter; Sequoia-Turner Corp., Mountain View, CA) and normalized for original LV myocardial sample weight (nmol His-Leu  $\text{mg LV}^{-1} \cdot \text{min}^{-1}$ ).

**Data Analysis.** Indices of LV function and systemic hemodynamics were compared among the treatment groups with ANOVA. If the ANOVA revealed significant differences, pairwise tests of individual group means were compared with Bonferroni probabilities. For comparisons of neurohormonal profiles and MMP zymographic activity, the Student-Newman-Kuels test was used. All statistical procedures were performed with the BMDP statistical software package (BMDP Statistical Software Inc., Los Angeles, CA). Results are presented as means  $\pm$  S.E. Values of  $p < .05$  were considered to be statistically significant.

## Results

After the 21-day pacing protocol, peak plasma concentrations of the MMP inhibitor (drawn 2–3 h postdose) were  $10.8 \pm 1.2 \mu\text{g/ml}$  in the MMP-inhibition-only group and were similar in the combined ACE and MMP inhibition group ( $11.7 \pm 1.9 \mu\text{g/ml}$ ). The trough plasma levels of the MMP inhibitor in the MMP-inhibition-only group were  $8.4 \pm 1.1 \mu\text{g/ml}$  and  $11.3 \pm 1.3 \mu\text{g/ml}$  in the combined treatment group; these plasma levels were not significantly different ( $p = .13$ ).

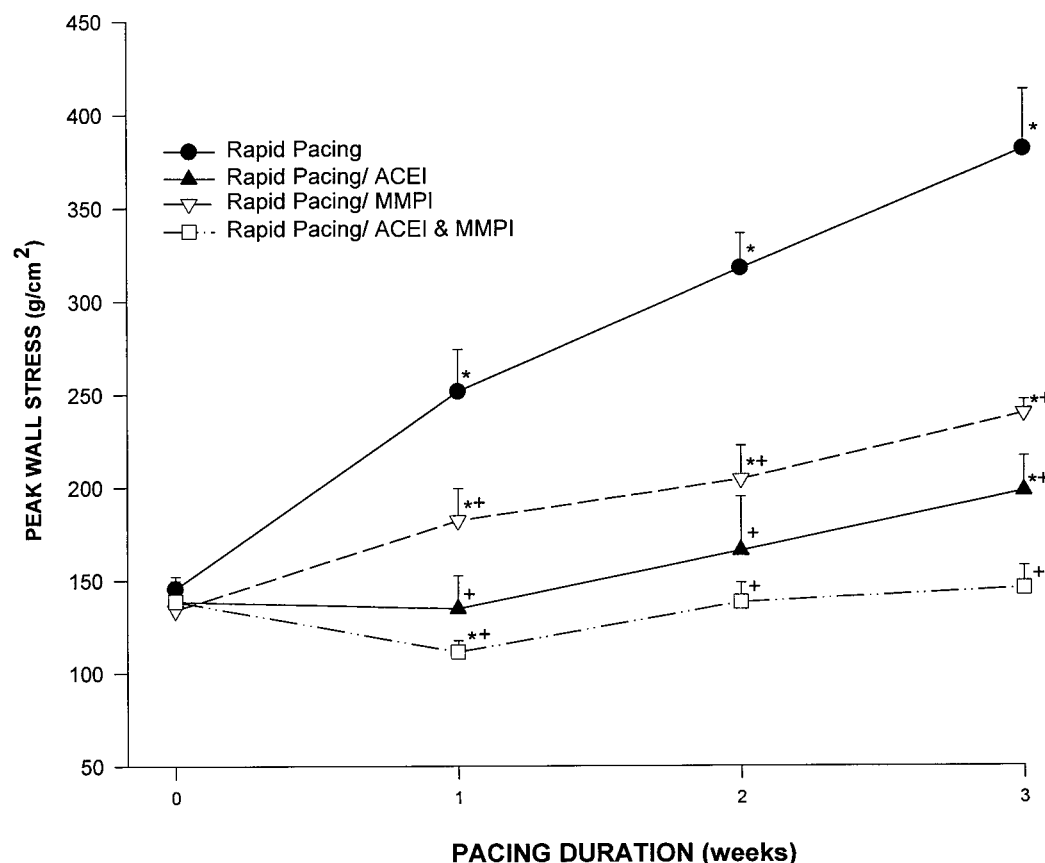
**LV Size and Function.** Weekly changes in LV size and function obtained in the conscious, awake state for the rapid pacing groups are summarized in Fig. 2. In the untreated pacing group, LV end-diastolic dimension and peak wall stress increased, and fractional shortening decreased in a time-dependent manner. In all treatment groups, LV end-diastolic dimension was reduced from untreated pacing values with each week of pacing. LV dimension was the lower in the combined inhibition group compared with MMP inhibition values. With MMP inhibition or ACE inhibition, LV peak wall stress remained unchanged from baseline values after 1 week of rapid pacing, but increased from baseline values after 3 weeks of pacing (Fig. 3). LV peak wall stress was significantly lower in the all treatment groups compared with the untreated pacing group. LV peak wall stress remained unchanged from baseline values in the combined inhibition group (Fig. 3). LV fractional shortening fell from baseline values in all treatment groups with rapid pacing, but after 3 weeks of rapid pacing, LV fractional shortening was higher in all treatment groups compared with rapid-pacing-only values. At 3 weeks of pacing, LV end-diastolic dimension to wall thickness ratio was doubled in the rapid-pacing-only group compared with controls ( $10.4 \pm 0.5$  versus  $4.2 \pm 0.2$ , respectively,  $p < .05$ ). In the MMP inhibition and



**Fig. 2.** LV size and function were serially measured with each week of rapid pacing with no treatment, with concomitant ACE inhibition (ACEI), MMP inhibition (MMPI), and combined inhibition (ACEI and MMPI). All measurements were performed with the pacemaker deactivated and in the conscious state. Top, LV end-diastolic dimension increased from baseline values after 1 week of pacing and increased in a time-dependent manner with longer durations of pacing. In all treatment groups, LV end-diastolic dimension was reduced from untreated pacing values with each week of pacing. LV end-diastolic dimension was the lower in the combined inhibition group when compared with MMP inhibition values ( $p < .05$ ). Bottom, LV fractional shortening fell in a time-dependent manner in all rapid-pacing groups. However, after 3 weeks of rapid pacing, LV fractional shortening was higher in the all treatment groups compared with rapid-pacing-only values ( $*p < .05$  versus time 0 baseline values;  $+p < .05$  versus pacing-only values).

ACE-inhibition-only groups, the LV end-diastolic dimension/wall thickness ratio was reduced to a similar degree ( $7.5 \pm 0.5$  and  $7.3 \pm 0.2$ , respectively) from untreated 3-week pacing values ( $p < .05$ ). In the combined treatment group, LV end-diastolic dimension/wall thickness ratio was reduced from single treatment values ( $6.3 \pm 0.04$ ,  $p < .05$ ) but remained increased from control values ( $p < .05$ ).

**LV Systolic and Diastolic Function.** Systemic hemodynamics in the anesthetized, instrumented animals is summarized in Table 1. In the untreated pacing group, cardiac output and LV systolic pressure were reduced and pulmonary and systemic vascular resistance increased from control values. In the ACE inhibition group, cardiac output, peak LV dP/dt, and pulmonary vascular resistance were similar to control values. In the ACE inhibition group, systemic vascular resistance was reduced from pacing-only values. With MMP inhibition, systemic hemodynamics was similar to untreated rapid pacing values, but LV end-diastolic pressure



**Fig. 3.** After 1 week of rapid pacing, LV peak wall stress increased from baseline values in the untreated group and increased from this value with longer durations of pacing. In the MMPi and ACEI groups, LV peak wall stress remained unchanged from baseline values after 1 week of rapid pacing, but increased from baseline values after 3 weeks of pacing. At each week of pacing, LV peak wall stress was significantly lower in the all treatment groups compared with the untreated pacing group. LV peak wall stress remained unchanged from baseline values in the combined inhibition group (\* $p < .05$  versus time 0 baseline values; + $p < .05$  versus pacing-only values).

TABLE 1

Systemic hemodynamics with chronic rapid pacing: Effects of chronic matrix metalloproteinase inhibition, ACE inhibition, or combined treatment. All values are means  $\pm$  S.E. Pulmonary artery pressure reported as mean value.

	Control	Rapid Pacing <sup>a</sup>	Rapid Pacing and ACEI <sup>b</sup>	Rapid Pacing and MMPi <sup>c</sup>	Rapid Pacing and ACEI/MMPi <sup>d</sup>
Resting heart rate (beats/min)	94 $\pm$ 9	144 $\pm$ 10*	118 $\pm$ 7†	135 $\pm$ 11*	132 $\pm$ 7*
Pump Function					
Stroke volume (ml)	35.34 $\pm$ 2.59	15.19 $\pm$ 1.62*	28.10 $\pm$ 2.33*†	16.74 $\pm$ 3.03*§	27.03 $\pm$ 4.17*†¶
Cardiac output (L/min)	3.20 $\pm$ .19	2.10 $\pm$ .14*	3.26 $\pm$ .23†	2.08 $\pm$ .27*§	3.44 $\pm$ 0.39†¶
Pressures					
Mean arterial pressure (mm Hg)	87 $\pm$ 2	79 $\pm$ 4	71 $\pm$ 4*	84 $\pm$ 5§	62 $\pm$ 4*†¶
LV Peak-systolic pressure (mm Hg)	120 $\pm$ 3	103 $\pm$ 4*	100 $\pm$ 5*	110 $\pm$ 3*	86 $\pm$ 4*†§¶
LV End-diastolic pressure (mm Hg)	9 $\pm$ 1	14 $\pm$ 1*	12 $\pm$ 2	18 $\pm$ 2*§	10 $\pm$ 2†¶
Pulmonary artery pressure (mm Hg)	16 $\pm$ 1	24 $\pm$ 2*	22 $\pm$ 2*	27 $\pm$ 2*§	16 $\pm$ 1†¶
Peak + dP/dt (mm Hg/s)	1960 $\pm$ 148	1322 $\pm$ 70*	1714 $\pm$ 117†	1409 $\pm$ 143*	1492 $\pm$ 172*
Resistances					
Systemic (dyne·s·cm <sup>-5</sup> )	1977 $\pm$ 100	2521 $\pm$ 194*	1529 $\pm$ 174*†	2784 $\pm$ 338*§	1310 $\pm$ 143*†¶
Pulmonary (dyne·s·cm <sup>-5</sup> )	159 $\pm$ 31	378 $\pm$ 66*	266 $\pm$ 68	417 $\pm$ 158*	178 $\pm$ 54†
Neurohormones					
Norepinephrine (pg/ml)	262 $\pm$ 28	963 $\pm$ 133*	618 $\pm$ 51*†	736 $\pm$ 161*	311 $\pm$ 61†§¶
Renin activity (ng/ml/h)	4.8 $\pm$ 0.5	24.2 $\pm$ 3.0*	8.4 $\pm$ 2.7†	10.6 $\pm$ 4.1†	12.1 $\pm$ 1.7*†
Sample size (n)	9	8	8	8	8

\* $p < .05$  versus control; † $p < .05$  versus rapid pacing only; § $p < .05$  versus rapid pacing and ACEI; ¶ $p < .05$  versus rapid pacing and MMP inhibition.

<sup>a</sup> Rapid pacing: 21 days of rapid pacing, 240 beats/min.

<sup>b</sup> Rapid pacing and chronic ACE inhibition: fosinopril, 2.5 mg/kg b.i.d. p.o.

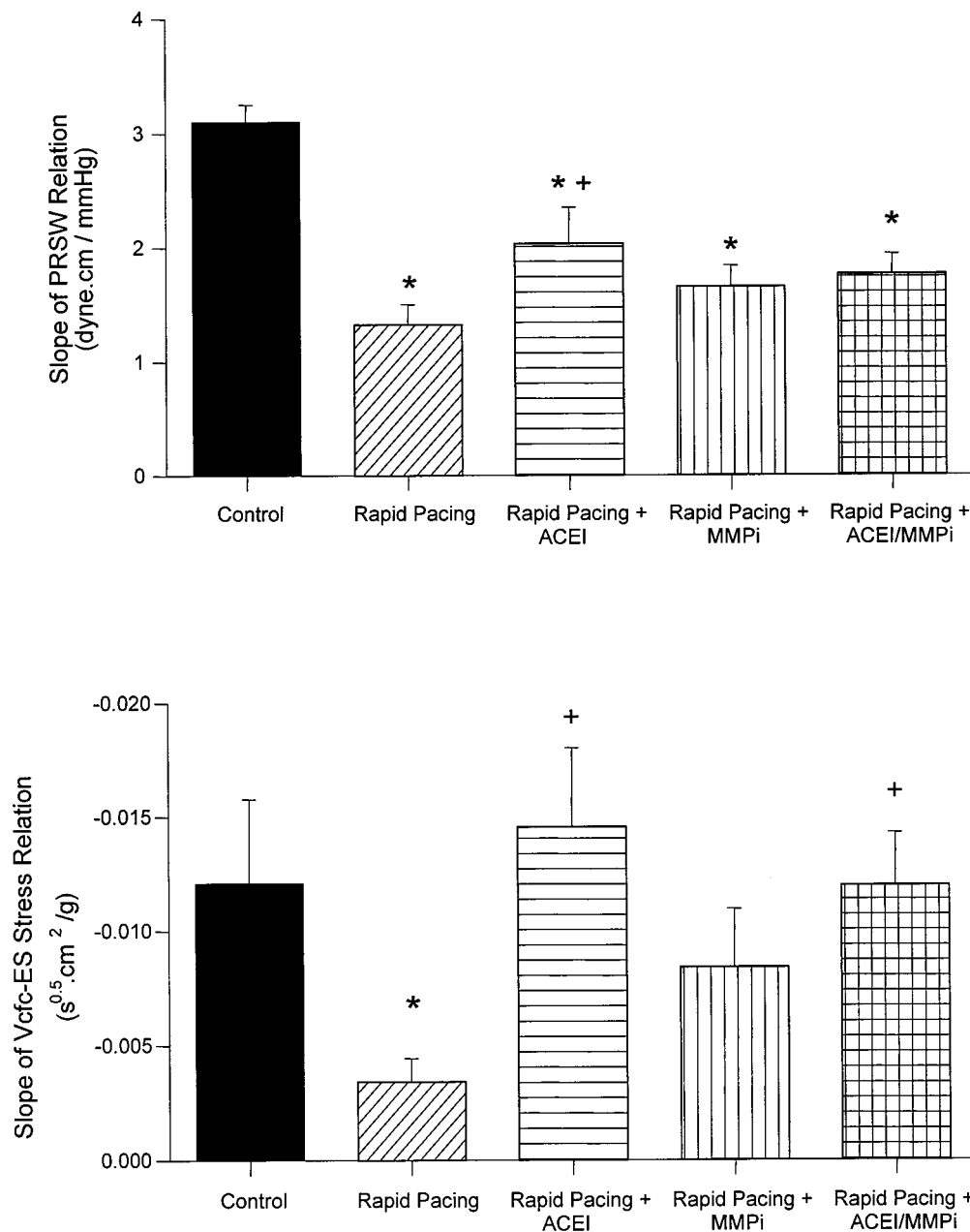
<sup>c</sup> Rapid pacing and chronic MMP inhibition: PD166793, 2 mg/kg s.i.d. p.o.

<sup>d</sup> Rapid pacing and chronic ACE inhibition/MMP inhibition: fosinopril, 2.5 mg/kg b.i.d. p.o.; PD166793, 2 mg/kg s.i.d. p.o.

was increased. With combined ACE and MMP inhibition, cardiac output and pulmonary vascular resistance were normalized. In the combined treatment group, mean arterial pressure, LV systolic pressure, and systemic vascular resistance were reduced from control values.

The slope of the LV PRSWR for the different treatment groups is summarized in Fig. 4. The slope of the PRSWR was reduced in all rapid pacing groups compared with control

values. In the ACE-inhibition-only group, the slope of the PRSWR was increased from rapid-pacing-only values. The slope of the Vcfc-stress relation was reduced from control values in the untreated rapid-pacing group, and it was increased from rapid-pacing-only values in the ACE inhibition and combination treatment groups. The chamber and myocardial stiffness constants were unchanged from control values with chronic rapid pacing but were both significantly increased



**Fig. 4.** Indices of LV ejection performance determined by alterations in preload after 3 weeks of chronic rapid pacing, with concomitant ACE inhibition (ACEI), MMP inhibition (MMPi), or combined inhibition (ACEI/MMPi). Top, the slope of the PRSW relation was reduced in all rapid-pacing groups when compared with control. In the ACEI group, the slope of this relation was increased compared with rapid-pacing-only values ( $p < .05$ ). Bottom, the slope of the velocity of circumferential fiber shortening, corrected for heart rate (Vcfc) and end-systolic (ES) wall stress relation was reduced after 3 weeks of rapid pacing. The slope of this relation was increased from untreated-pacing values in the ACEI and combined treatment groups and was not different from control values. In the MMPi-only group, the slope of this relationship was not statistically significant from control ( $p = .35$ ) or from rapid-pacing-only values ( $p = .15$ ). \* $p < .05$  versus control; + $p < .05$  versus rapid-pacing-only.

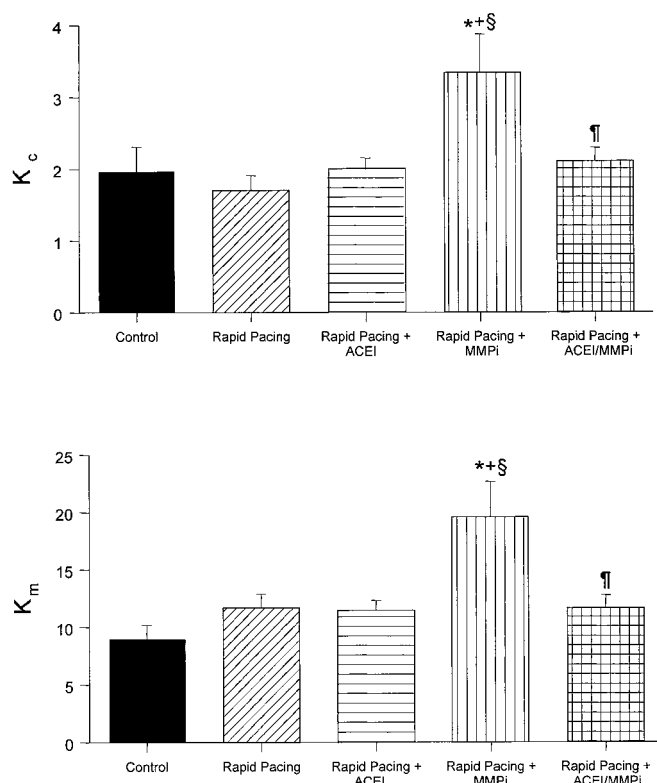
in the MMP inhibition group (Fig. 5). With combined ACE and MMP inhibition during chronic pacing, both chamber and myocardial stiffness were unchanged from controls.

**Plasma Neurohormones.** Plasma norepinephrine and renin activity increased by  $>3$ -fold in the chronic rapid-pacing group compared with controls (Table 1). In the rapid-pacing and ACE inhibition group, plasma norepinephrine was reduced from rapid-pacing-only values but remained increased from controls. In the rapid-pacing and MMP inhibition group, plasma norepinephrine was similar to rapid-pacing-only values, but renin activity was decreased. In the combination treatment group, plasma norepinephrine and renin activity was decreased from rapid-pacing-only values.

**LV Myocardial Morphometry.** The relative content of LV myocardial fibrillar collagen was reduced in the rapid-pacing-only group compared with control values ( $2.08 \pm 0.05$  versus  $1.53 \pm 0.02\%$ ,  $p < .05$ ) and remained reduced in the

rapid-pacing and ACE inhibition group ( $1.46 \pm 0.09\%$ ,  $p < .05$ ). In the rapid-pacing and MMP inhibition group, fibrillar collagen density was similar to control values ( $1.91 \pm 0.32\%$ ). In the combination treatment group, myocardial fibrillar density was reduced from control ( $1.67 \pm 0.04\%$ ,  $p < .05$ ) and was similar to rapid-pacing-only values. Representative scanning electron micrographs are shown in Fig. 6. In the control myocardium, the fibrillar collagen weave could be readily appreciated between myocytes. In the rapid-pacing-only group, significant disruption of the collagen weave with areas devoid of fibrillar collagen could be readily appreciated. In the rapid-pacing and ACE inhibition group, a fine fibrillar weave could be observed between myocytes but this appeared reduced from control myocardium. In the rapid-pacing and MMP inhibition group, a fibrillar collagen weave and collagen struts could be easily observed between myocytes, and in regions, appeared increased from control myocardium. In the





**Fig. 5.** The LV chamber stiffness ( $K_c$ , top) and myocardial stiffness ( $K_m$ , bottom) constants were determined through the analysis of isochronal measurements of LV end-diastolic dimensions and pressures. In the rapid-pacing-only group and the ACE inhibition group (ACEI), there were no changes in either chamber or myocardial stiffness compared with controls. However, with MMP inhibition (MMPi), both stiffness constants were increased from both control and pacing-only values. Combined ACEI and MMPi with rapid pacing reduced chamber and myocardial stiffness constants from MMPi-only values and were similar to control values ( $^*p < .05$  versus control;  $^+p < .05$  versus rapid-pacing only;  $^§p < .05$  versus ACEI;  $^¶p < .05$  versus MMPi).

combination treatment group, the fibrillar collagen weave could be readily appreciated between myocytes, and in regions, appeared to be similar to control myocardium.

**LV Myocardial Zymographic and ACE Activity.** In LV myocardial extract preparations, MMP zymographic activity was examined with gelatin as the proteolytic substrate. MMP gelatinolytic activity was increased in LV myocardial extracts from the rapid-pacing group compared with control values ( $10,923 \pm 2052$  versus  $17,302 \pm 2837$  pixels,  $p < .05$ ). In all treatment groups, zymographic activity was unchanged from control values. In normal porcine LV myocardial extract preparations, MMP zymographic activity was reduced by  $>50\%$  in the presence of  $3 \mu\text{g/ml}$  PD166793 ( $p < .05$ , results from three independent experiments). MMP zymographic activity was reduced to below detectable levels when concentrations of  $10 \mu\text{g/ml}$  PD166793 were included in the proteolytic assay system.

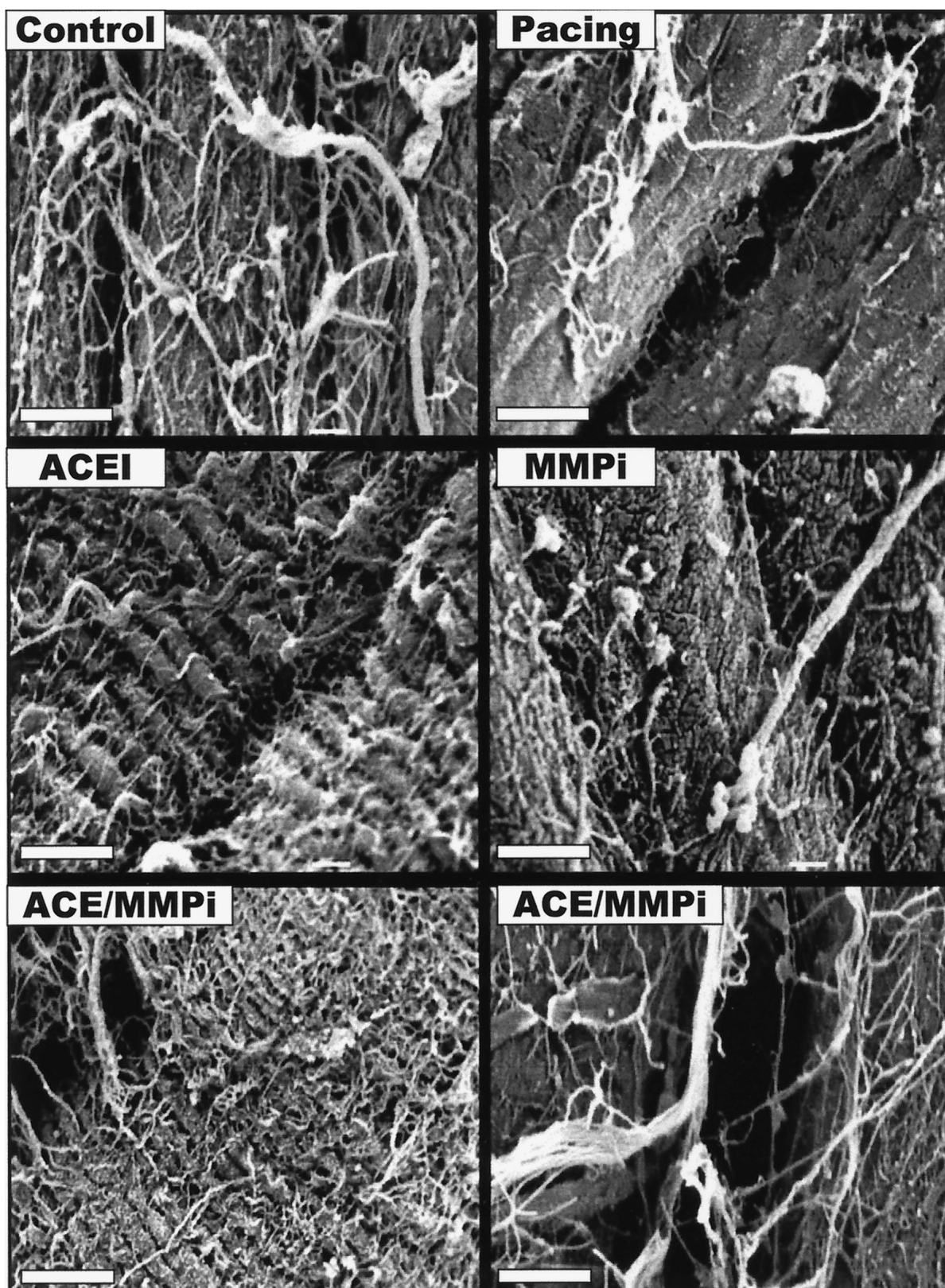
LV myocardial ACE activity was reduced in the rapid-pacing-only group compared with control values ( $810 \pm 44$  versus  $670 \pm 52 \text{ nmol mg LV}^{-1} \cdot \text{min}^{-1}$ ,  $p = .06$ ). Reduced in vitro LV myocardial ACE activity has been reported previously with chronic rapid pacing (Spinale et al., 1997). In all treatment groups, LV myocardial ACE activity was unchanged from control values.

**Specificity of MMP Inhibition.** Based on the observations that MMP inhibition influenced LV geometry and function, and that in combination with ACE inhibition significantly influenced systemic hemodynamics, a more careful analysis of the properties of the MMP inhibitor used in the present study was undertaken. The MMP inhibitor chosen for these studies has a chemical formulation of (S)-2-(4'-bromo-biphenyl-4-sulfonylamino-3-methyl) butyric acid and has global MMP inhibitory activity at the 8 to  $10 \mu\text{M}$  range based on in vitro assay systems (Table 2). Specifically, with full-length purified human MMPs, this MMP inhibitor demonstrated inhibitory activity against the gelatinases (MMP-9,2), the stromelysins (MMP-3,7), and interstitial collagenase (MMP-1) with an artificial substrate system described in detail previously (Ye et al., 1992, 1994, 1995). This MMP inhibitor also was examined with respect to activity against other proteolytic systems. Specifically, activity against ACE (prepared from rabbit lung) (Bunning et al., 1983), neutral endopeptidase (24.11, membrane fraction from Burkitt lymphoma cell line) (Shipp et al., 1989), endothelin-converting enzyme (membrane fraction from Chinese hamster ovary cells transfected with human endothelin converting enzyme 1) (Ahn et al., 1995), and tumor necrosis factor  $\alpha$  convertase (tumor necrosis factor- $\alpha$  release from stimulated leukocytes; PanLabs Inc., WA) (Welker et al., 1996). Concentrations of up to  $100 \mu\text{M}$  PD166793 did not exhibit inhibitory activity against these proteolytic systems (Table 2).

## Discussion

Pharmacological interventions such as ACE inhibition have been demonstrated to provide favorable effects on LV remodeling and slow the progression to LV failure (Konstam et al., 1993; Greenberg et al., 1995). These observations would suggest that strategies that will slow the myocardial remodeling process would be important therapeutic strategies in the setting of developing CHF. The activation and expression of the MMPs have been implicated to be involved in a number of tissue-remodeling processes (Hansen-Birkedal et al., 1993; Rees et al., 1993; Werb et al., 1993; Dollery et al., 1995). Recent studies have demonstrated increased myocardial MMP activity in both clinical and animal models of CHF (Gunja-Smith et al., 1996; Spinale et al., 1998; Thomas et al., 1998). However, these studies provide only circumstantial evidence that MMP activation contributes to the changes in LV geometry with developing CHF. Accordingly, the present study examined the comparative effects of ACE inhibition, MMP inhibition, and combined inhibition on LV systolic and diastolic function with the development of pacing-induced CHF. There were several unique and important findings of the present study. First, MMP inhibition significantly reduced the degree of LV dilation that invariably occurs with pacing CHF, but this was accompanied by increased chamber and myocardial stiffness properties. Second, ACE inhibition reduced the degree of LV dilation similar to that of MMP inhibition, but this was not associated with increased LV stiffness properties. Third, combined ACE and MMP inhibition with rapid pacing significantly reduced LV wall stress and improved LV pump function. Fourth, ACE inhibition combined with MMP inhibition prevented the increased chamber and myocardial stiffness that was observed with MMP inhibition alone. The results suggest that in-





**Fig. 6.** Representative scanning electron micrographs of LV myocardial sections taken from control myocardial preparations, following chronic rapid pacing (Pacing), chronic pacing with concomitant ACE inhibition (ACEI), chronic pacing with concomitant MMP inhibition (MMPi), and chronic pacing with concomitant ACE and MMP inhibition (ACE/MMPi). In control myocardium, the collagen matrix could be readily observed to surround individual myocytes in a homogenous and weave-like pattern. After 3 weeks of rapid pacing, the collagen weave appeared to be significantly disrupted, with large areas of discontinuity between myocytes. With concomitant ACE inhibition, the fibrillar collagen weave could be observed but was reduced. In the MMP-inhibition-only group, the collagen weave appeared to be thickened between myocytes. The lower panels present two scanning micrographs for the combined ACE and MMP inhibition group. In some areas, a fibrillar collagen weave was readily appreciated in the interstitial space. In other regions, the fibrillar collagen weave appeared to be disrupted from normal architecture. White bar, 2  $\mu$ m.

TABLE 2

Protease inhibitory activity of the matrix metalloproteinase MMP inhibitor PD 166793

Enzyme Classification		IC <sub>50</sub> <sup>a</sup> μM
MMP <sup>b</sup>		
Interstitial collagenase		
MMP-1		6.100
Stromelysins		
MMP-3		0.012
MMP-7		8.100
Gelatinases		
MMP-2		0.047
MMP-9		9.900
ACE	>100 (n/c) <sup>c</sup>	
Neutral endopeptidase		>100 (n/c) <sup>c</sup>
Endothelin-converting enzyme	>100 (n/c) <sup>c</sup>	
Tumor necrosis factor α-converting enzyme		>100 (n/c) <sup>c</sup>

<sup>a</sup> Data expressed as concentration necessary to inhibit 50% of maximal enzyme activity.

<sup>b</sup> MMP activity assays determined with recombinant human MMP constructs of the catalytic domain and an artificial substrate assay system.

<sup>c</sup> PD166793 was not active at up to 100 μM and therefore a true IC<sub>50</sub> was not computed (n/c).

creased MMP activation contributes to the LV dilation that occurs in this model of pacing CHF and that a downstream event with ACE inhibition may be modulation of MMP activity. Furthermore, ACE and MMP inhibition may have interactive effects with respect LV stiffness properties with CHF.

This is the first study that examined the comparative effects of ACE inhibition and MMP inhibition in the setting of developing CHF. Both ACE inhibition and MMP inhibition blunted the degree of LV dilation with pacing CHF. Because an important determinant of LV wall stress is chamber dimension, LV peak wall stress was reduced in both the ACE and MMP inhibition groups compared with untreated pacing-CHF values. The beneficial effect of either ACE or MMP inhibition on LV geometry, and therefore on LV wall stress, was a likely contributory factor for the improved LV fractional shortening that was observed in both of these treatment groups. A contributory factor for the changes in LV wall stress patterns in the rapid-pacing groups that occurred in the present study was the ratio of LV dimension/wall thickness. Specifically, the development of pacing CHF is accompanied by increased LV chamber volumes and a reduction in posterior wall thickness (Komamura et al., 1993; Spinale et al., 1991a,b, 1995, 1997, 1998). In the present study, LV wall stress was reduced with MMP inhibition primarily by a preservation of LV wall thickness as well as by a reduction in end-diastolic dimension compared with untreated CHF values. With ACE inhibition, the reduction in LV wall stress was primarily due to a reduction in the degree of LV dilation and systolic pressure. Finally, combined treatment with rapid pacing influenced all of the contributory factors used to compute LV wall stress. Specifically, LV systolic dimension and pressure were reduced and wall thickness increased to the greatest degree compared with untreated pacing-CHF values. Thus, combined treatment appeared to exert the most effect on LV remodeling with respect to a reduction in LV wall stress patterns.

In addition to the reduction in the degree of LV dilation, mean arterial pressure was significantly reduced in the combined ACE and MMP inhibition group, which translated into the greatest reduction in LV peak wall stress. In the anes-

thetized and fully instrumented preparation, cardiac output and systemic hemodynamics were improved in the ACE inhibition group but not with MMP inhibition. The beneficial effect of ACE inhibition on hemodynamic profiles in this model of pacing CHF is consistent with past reports (Spinale et al., 1995, 1997). There have been several past reports that have implicated the MMPs in vascular remodeling (Dollery et al., 1995). Thus, in the present study, chronic MMP inhibition may have altered vascular structure and composition, which in turn would negatively influence vascular compliance characteristics. Because MMP inhibition did not reduce systemic or pulmonary vascular resistance in this model of pacing CHF, a future study that is focused on the effects of chronic MMP inhibition on the structure and function of the peripheral vasculature would be appropriate.

In the present study, the plasma levels of MMP inhibitor that were obtained exceeded the levels necessary to achieve significant inhibitory effects against a number of species of MMPs (Table 2). Moreover, the MMP inhibitor significantly attenuated MMP zymographic activity in LV myocardial extracts at plasma concentrations achieved in the present study. However, it must be recognized that this analysis was predicated on in vitro assay systems and may not actually reflect MMP inhibitory activity at the tissue level in vivo. Nevertheless, concomitant MMP inhibitor treatment with rapid pacing significantly affected certain indices of LV geometry consistent with a biological response to this pharmacological regimen. One of the more interesting and unique outcomes from the present study was the apparent additive/synergistic effects of ACE and MMP inhibition on arterial pressure and systemic vascular resistance. Based on the initial Ang-I pressor studies, MMP inhibition did not appear to potentiate the effects of ACE inhibition. However, the profound vasodilatory effects that were observed with combined ACE and MMP inhibition suggest that some interactive effects occurred with respect to influencing neurohormonal pathways that modulate vascular smooth muscle tone. Because combined ACE and MMP inhibition significantly reduced LV loading conditions, this was an important contributory mechanism for the effects on LV geometry and function. One strategy to differentiate the effects of systemic loading conditions to that of local myocardial effects on the LV remodeling process would be to use other pharmacological strategies that reduce systemic vascular resistance without influencing myocardial neurohormonal and proteolytic systems. In light of the findings from the present study, then future studies that more carefully address this issue would be appropriate.

In the present study, combined treatment did not appear to affect the clearance rates or pharmacokinetics of the MMP inhibitor (as evidenced by no change in peak or trough levels). In the LV myocardial assay systems, combined treatment did not appear to influence MMP activity to a greater degree than that observed with either treatment alone. In the present study and consistent with a past report, in vitro myocardial ACE activity was reduced with pacing CHF (Spinale et al., 1997). The LV remodeling process that occurs with pacing CHF is similar to the clinical forms of dilated cardiomyopathy in which LV volumes increase significantly without a proportional increase in LV wall thickness and mass. A past clinical study reported a 50% reduction in LV myocardial ACE activity in end-stage dilated cardiomyopa-



thy compared with normal values (Urata et al., 1990). Consistent with past observations (Spinale et al., 1997), the present study demonstrated that with chronic ACE inhibition, *in vitro* myocardial ACE activity was increased from pacing-CHF values. Taken together, these observations imply that a local feedback loop exists with respect to the myocardial ACE system. In the present study, MMP inhibition also increased myocardial ACE activity from untreated pacing values. Characterization of the MMP inhibitor used in the present study demonstrated that this compound possessed no direct activity against other enzymatic systems that have been identified to be relevant in the CHF process. However, these assays were performed under highly controlled *in vitro* conditions. Whether chronic MMP inhibition influences other myocardial proteolytic systems remains to be established. It is likely that advancements in the pharmacological interventions for the treatment of CHF will probably be instituted with a background of ACE inhibition. Thus, the underlying mechanisms for the effects of combined MMP and ACE inhibition with respect to systemic vascular resistive properties and alternative enzymatic pathways warrant further investigation.

In the present study, significant changes in LV geometry and loading conditions occurred with pacing CHF and with the different treatment interventions, which can make assessment of LV ejection performance difficult. To address this issue, two indices of LV ejection performance were evaluated: the PRSWR and the rate-corrected Vcfc end-systolic-stress relation. Both of these indices of LV ejection performance were significantly reduced with pacing CHF. This laboratory has reported previously that the development of pacing-induced CHF is accompanied by a significant reduction in LV myocyte contractile performance and inotropic responsiveness (Spinale et al., 1995, 1998). Thus, the reduction in both of these indices of LV ejection performance was due, at least in part, to a reduction in myocardial contractility. While remaining reduced, concomitant ACE inhibition with rapid pacing improved these indices of LV ejection performance from untreated pacing values; which suggests an inherent improvement in LV myocardial performance. With MMP inhibition alone, the PRSWR relation was unchanged from untreated pacing-CHF values, whereas the Vcfc-stress relation was somewhat improved. With combined ACE and MMP inhibition, the Vcfc-stress relation was significantly improved from pacing-CHF values; again suggesting an intrinsic improvement in LV myocardial performance. A likely contributory mechanism for the improvement in the indices of LV ejection performance with ACE inhibition monotherapy or in combination with MMP inhibition was the reduction in sympathetic activation as evidenced by a reduction in plasma norepinephrine. It has been demonstrated previously that ACE inhibition in this model of pacing CHF reduced plasma norepinephrine and was associated with an intrinsic improvement in myocyte contractile function (Spinale et al., 1995). Thus, the increased slope of the Vcfc-stress relation with combination treatment was probably due to an inherent improvement in myocardial contractility. However, it must be recognized that although these indices of LV ejection performance are relatively load-independent, significant limitations exist with both approaches. The limitations of the PRSWR include the fact that LV end-diastolic pressure reflects intracavitary pressure and therefore can be influ-

enced by transmural and external pressure and that this relationship is nonlinear at high end-diastolic pressures (Kass et al., 1989). In the present study, all PRSWR measurements were derived with the chest open and the pericardium incised. However, LV end-diastolic pressure was increased in the pacing-CHF group and was significantly increased in the MMP inhibition group. Although the PRSWR reflects the capacity of the LV to perform work at a given load, it can be influenced by arterial elastance (Glomer et al., 1985). Thus, differences in arterial elastance may have occurred in the present study with ACE or MMP inhibition that would influence the slope of the PRSWR.

Because changes in the myocardial collagen matrix have been implicated to influence LV compliance (Weber et al., 1992), an important objective of the present study was to examine LV stiffness characteristics following the different treatment interventions. With the development of pacing CHF, there were no significant changes in either the LV chamber or myocardial stiffness constants compared with controls. These findings are consistent with past studies from this laboratory that have demonstrated that pacing CHF is not accompanied by significant changes in LV stiffness characteristics from control animals when examined under equivalent LV end-diastolic pressures (Tomita et al., 1991; Komamura et al., 1993). With concomitant ACE inhibition during rapid pacing, the relative reduction in LV chamber dimensions was not associated with changes in these LV stiffness constants. In marked contrast, MMP inhibition caused a significant increase in both chamber and myocardial stiffness. An increase in LV chamber stiffness will have important consequences on LV filling characteristics during diastole. A number of factors influence LV chamber stiffness, such as loading conditions, myocardial active relaxation processes, and myocardial stiffness. The findings from the present study demonstrated that the increased LV chamber stiffness with MMP inhibition was probably due to an absolute increase in LV myocardial stiffness. Because LV myocardial stiffness reflects intrinsic material properties of the myocardium itself, this increase in myocardial stiffness with MMP inhibition was probably due changes in myocardial collagen content and structure (Spinale et al., 1991a). This conclusion is further supported by the fact that the relative content of LV myocardial fibrillar collagen content was increased from pacing-CHF values with MMP inhibition. Thus, although 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. However, an important and significant finding of the present study was that combined ACE and MMP inhibition did not cause any change in LV stiffness characteristics. This laboratory has previously demonstrated that ACE inhibition during chronic rapid pacing attenuated the changes in myocardial collagen architecture (Spinale et al., 1995). Furthermore, ACE inhibition has been demonstrated to provide favorable effects on LV myocyte geometry (Spinale et al., 1995). In the present study, the relative content of LV myocardial fibrillar collagen content was not increased from pacing-CHF values with combination treatment. However, scanning electron microscopy revealed an improved structural homogeneity of the collagen weave in this combination treatment group. Thus, combined ACE and MMP inhibition may work in an additive fashion by

preserving myocyte and myocardial fibrillar architecture, respectively.

In the present study, zymographic activity was increased with pacing CHF, indicating increased abundance of certain species of MMPs occurred (Spinale et al., 1998; Thomas et al., 1998). With either ACE or MMP inhibition, relative MMP zymographic activity was not altered from normal control values. However, zymographic analysis requires stringent myocardial extraction procedures and electrophoretic separation. Whether changes in LV myocardial activity with either ACE or MMP inhibition occurred in vivo cannot be determined through this approach. A number of species of MMPs have been described with reported differences in substrate specificity and differing abundance in various tissues (Hansen-Birkedal et al., 1993; Rees et al., 1993; Dollery et al., 1995). For example, stromelysin, or MMP-3, has been shown to activate other MMPs as well as to have affinity for a number of extracellular matrix proteins (Nagase, 1997). More importantly, MMP-3 has been demonstrated to be selectively increased in human cardiomyopathy, whereas MMP-1 appears to be down-regulated (Thomas et al., 1998). Thus, global nonselective MMP inhibition, as used in the present study, may not be the most effective approach in modulating MMP activity and matrix degradation in developing CHF. Nevertheless, the present study provides proof of concept that MMP activation contributes to the LV remodeling process in this model of CHF. However, whether the induction of MMPs is a primary event within the myocardial compartment that directly contributes to the LV remodeling with CHF or is a secondary phenomenon in this process remains to be established.

MMP activity is tightly controlled in normal myocardium by a family of closely related inhibitors known as tissue inhibitors of MMPs, or TIMPs (Hansen-Birkedal et al., 1993; Rees et al., 1993; Werb et al., 1993; Dollery et al., 1995). More recently, alterations in TIMP expression have been reported in human cardiomyopathic disease (Li et al., 1998; Thomas et al., 1998). The *MMP* and *TIMP* genes contain response elements in the promoter regions that will bind a number of transcription factors, such as activator protein 1 (AP-1) (Rees et al., 1993). Ang-II formation and subsequent Ang AT<sub>1</sub> receptor activation causes the activation of a number of kinases, which in turn may increase the formation of transcriptional factors such as AP-1 (Sadoshima et al., 1993). There are significant differences in the upstream response elements for the *MMP* and *TIMP* genes, including AP-1 (Edwards et al., 1996). Thus, ACE inhibition may influence expression of both MMPs and TIMPs in developing CHF. Although remaining speculative, combined MMP and ACE inhibition may influence overall MMP activity through two independent mechanisms: modulation of MMP activation directly and through changes in the expression of TIMP levels, respectively.

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