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Myocardial Interstitial Matrix Metalloproteinase Activity Is Altered by Mechanical Changes in LV Load

Interaction With the Angiotensin Type 1 Receptor

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Abstract—LV myocardial remodeling is a structural hallmark of hypertensive hypertrophy, but molecular mechanisms driving this process are not well understood. The matrix metalloproteinases (MMPs) can cause myocardial remodeling in chronic disease states, but how MMP activity is altered with a mechanical load remains unknown. The present study quantified interstitial MMP activity after a discrete increase in LV load and dissected out the contributory role of the angiotensin II Type 1 receptor (AT₁R). Pigs (38kg) were randomized to undergo (1) increased LV load by insertion of an intra-aortic balloon pump (IABP) triggered at systole for 3 hours, then deactivated (n=11); (2) IABP and AT₁R blockade (AT₁RB; valsartan, 3 ng/kg/hr; n=6). MMP activity was directly measured in the myocardial interstitium using a validated inline digital fluorogenic microdialysis system. IABP engagement increased LV peak pressure from 92±3 to 113±5 and 123±7 mm Hg in the vehicle and AT₁RB group, respectively, and remained elevated throughout the IABP period ($P<0.05$). With IABP disengagement, segmental shortening (% change from baseline of 0) remained depressed in the vehicle group (-32.2±11.8%, $P<0.05$) but returned to baseline in the AT₁RB group (2.3±12.5%). MMP activity decreased with IABP in both groups. At IABP disengagement, a surge in MMP activity occurred in the vehicle group that was abrogated with AT₁RB (3.03±0.85 versus 0.07±1.55 MMP units/hr, $P<0.05$). A transient increase in LV load caused a cyclic variation in interstitial MMP activity that is regulated in part by the AT₁R. These temporally dynamic changes in MMP activity likely influence myocardial function and structure with increased LV load. (*Circ Res.* 2005;96:1110-1118.)

Key Words: matrix metalloproteinase ■ extracellular matrix ■ angiotensin II type I receptor

Left ventricular (LV) hypertrophy is the primary remodeling process in the myocardium in response to a pressure overload.¹⁻³ Systemic hypertension is the most common cause of LV pressure overload and therefore incites significant LV remodeling.⁴ Structural events in this LV remodeling process secondary to hypertension include changes in the structure and composition of the extracellular matrix (ECM).¹⁻³ However, the underlying molecular determinants that drive the ECM remodeling process in hypertension-induced hypertrophy remain poorly understood. One important pathway in the changing of ECM structure and composition is activation of a family of proteases termed matrix metalloproteinases (MMPs).² In vitro studies have demonstrated that changes in stress and strain patterns can induce MMPs.⁵ Whether and to what degree mechanical stimuli, such as the in vivo induction of acute LV pressure overload, induce MMP activation has not been directly studied. Accordingly, the

central hypothesis of the present study was that changes in MMP activity occur during the induction and termination of acute LV pressure overload.

One aspect of systemic hypertension is the cyclic pattern of blood pressure changes over time.⁶ How these acute cyclic changes in systemic pressures may influence MMP induction and activation remains unknown. Whereas mechanical factors influence ECM structure and function with hypertension, biological mediators also clearly contribute to this process.⁷ Specifically, activation of the angiotensin II type 1 receptor (AT₁R) has been shown to occur with hypertension and influence ECM synthesis.^{8,9} Furthermore, the elaboration of angiotensin II (ang II) and subsequent activation of the AT₁R has been shown to modify MMP synthesis and release.⁹ Thus, the objectives of this study were to 1) directly interrogate the myocardial interstitium and quantify MMP activity during the induction and termination of acute LV pressure overload, and 2) to dissect out the effects of AT₁R activity with respect to

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MMP activation on induction and termination of an acute LV pressure overload.

The majority of past studies have examined MMPs indirectly or by extension.^{5,10–13} Specifically, biochemical methods have been used to measure MMP levels *ex vivo* and extrapolate these findings to the *in vivo* condition. However, MMP activity is a tightly regulated process that entails activation and endogenous inhibition.^{14,15} Therefore, extraction of myocardial tissue for MMP assays and directly determining MMP activity is problematic. Accordingly, the present study developed a novel system to directly quantitate MMP activity in the myocardial interstitium in real-time and thereby allow measurement of MMP activity *in vivo* after induction and termination of acute LV pressure overload.

Materials and Methods

Yorkshire pigs ($n=17$, 35 to 40 kg, Hambone Farms, Orangeburg, SC) were used for this 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" (National Research Council, Washington, 1996), and the Institutional Animal Care and Use Committee approved the protocol.

Instrumentation

Pigs were sedated with diazepam (200 mg po) and general anesthesia was induced using inhaled isoflurane (2%), sufentanyl ($2 \mu\text{g}/\text{kg}$ IV), etomidate ($0.3 \text{ mg}/\text{kg}$ IV), and vecuronium (10 mg IV) through an ear vein. The animal was then placed on the operating table, an open tracheostomy was performed, and mechanical ventilation was initiated to maintain arterial CO_2 between 35 to 45 mm Hg and pH 7.35 to 7.45. The right common carotid artery was accessed to transduce arterial blood pressure, and the right internal jugular vein was cannulated for maintenance of anesthesia and fluids (0.9% normal saline, 10 cc/kg/h). A balloon-tipped multi lumen thermodilution catheter (7.5 F, Baxter Healthcare Corp) was placed in the pulmonary artery via the left external jugular vein. The bladder was instrumented with a catheter for monitoring of urine output.

Before entering the chest, animals were premedicated with lidocaine (100 mg IV followed by 10 mg/h infusion), bretyllium (250 mg IV), and magnesium sulfate (2 g IV). Median sternotomy was performed and a precalibrated microtipped pressure transducer (7.5 F, Millar Instruments Inc) was placed through the apex of the LV through a stab incision and sutured into place. Next, 4 sonomicrometry crystals (2 mm, Sonometrics, Ontario) were placed at the endocardial surface of the apex, the LV mid-posterior wall, the anterior wall between the first and second diagonal branches of the left anterior descending artery, and on the epicardial surface of the anterior wall directly overlying the endocardial crystal in that location. A pacemaker lead was sutured to the left atrium, and the animals were atrially paced throughout the procedure at a target rate of 125 bpm. Through a purse-string suture in the ascending aorta or through the left internal iliac artery, an intra-aortic balloon catheter (IABP, Datascope, Montvale, NJ) was positioned in the descending thoracic aorta a minimum of 4 centimeters from the diaphragmatic division of the aorta. This approach did not affect hemodynamics or cardiac output. The animal was given heparin (150 U/kg IV) before placement of the balloon pump. Finally, 2 microdialysis catheters were inserted into the mid-myocardium of the anterior wall of the LV through a break-away sheath and connected to a custom-made system to measure real-time MMP activity, as described in detail below.

Microdialysis

Microdialysis probes with a molecular weight cutoff of 20 kDa and an outer diameter of 0.5 mm were placed in the LV. The molecular weight cutoff of the microdialysis probe prevented any MMP species from traversing the membrane. A fluorogenic substrate specific for

MMP-1, 2, 3, 7, and 9 (Calbiochem, La Jolla, Calif) at a concentration of $60 \mu\text{mol}/\text{L}$ was stored in a 2.5 mL Bee Stinger Gastight Syringe (Bioanalytical Systems, Inc) and infused at a rate of $5 \mu\text{L}/\text{min}$ through the 2 probes by the Baby Bee Syringe Drive controlled by the MD-1020 Bee Hive Controller (Bioanalytical Systems, Inc). Fluorescence emitted via substrate cleavage determined MMP activity. Substrate was allowed to equilibrate for 20 minutes before the beginning of the protocol. All tubing and syringes were protected from light. The specificity of this substrate was determined and performed as described for these fluorogenic substrates previously.^{16,17} Specifically, this substrate was incubated with MMP-2/9 recombinant protein (500 ng/mL, Chemicon) and demonstrated steady-state fluorescence emission by 60 minutes. With the addition of a broad spectrum MMP inhibitor (BB-94, 10 mmol/L, British Biotech), fluorescence emission was abolished.

Real-time Activity

One of the 2 probes was used to determine real-time MMP activity during the protocol. The dialysate that returned through the probe was connected to a FIALab PMT-FL Detector (FIALab Instruments, Inc). The dialysate traveled into the fluorescence detector where an UV light (Model D-1000CE, Analytical Instruments Systems, Inc, Flemington, NJ) and a 280 nm excitation filter excited the sample. The light then passed through a 360 nm 10 nm bandpass emission filter into the photomultiplier tube. The fluorescence detector was connected to a laptop computer and software (FIALab ver. 5) allowed for the real-time detection of changes in fluorescence.

Cumulative Activity

The remaining probe was used to validate this novel real-time activity detection system. Dialysate returning from this probe was collected into amber microcentrifuge tubes at 30-minute intervals throughout the protocol. After each interval, the tube was stored on ice. On completion, 100 μL of each dialysate sample was added to a 96-well polystyrene plate (Nalge Nunc International) and read at an excitation wavelength of 280 nm and an emission wavelength of 360 nm on the FLUOstar Galaxy fluorescent microplate reader (BMG Labtechnologies, Durham, NC).

Protocol

After instrumentation was completed and the microdialysis catheters were equilibrated, a 30-minute baseline period was begun. At the end of the baseline time point, a full hemodynamic profile was obtained, including measurement of LV and aortic pressures, pulmonary pressures, pulmonary capillary wedge pressure (PCWP), cardiac output (in triplicate), and urine output. In addition, blood was collected for measurement of plasma renin activity (PRA) as described previously.^{18,19} In a subset of animals, LV myocardial biopsies were taken at baseline, after the 180 minutes of IABP engagement, and at the end of the protocol. MMP fluorogenic substrate was collected from both microdialysis catheters. Pressure waveforms and sonomicrometry crystal signals were digitized on computer for subsequent analysis at a sampling frequency of 100 Hz (Sonolab, Sonometrics).

After baseline measurements, pigs were randomized to an angiotensin receptor blocker (AT_1RB , Valsartan) or vehicle (normal saline). The AT_1RB was given as a bolus of 1 mg/kg over 1 to 2 minutes, followed by an infusion of 3 ng/kg/h, which was continued throughout the protocol. This dose was previously found to have no effect on mean arterial pressure, yet still inhibited the angiotensin pressor response.²⁰

After randomization, the intra-aortic balloon pump was activated and augmented so that the balloon inflated during systole and deflated during diastole, creating a noticeable increase in LV peak pressure as seen on the pressure waveform. Hemodynamic and sonomicrometry crystal tracings were digitally recorded at 30-minute intervals for 180 minutes along with blood and microdialysis effluent collection. The balloon pump was then disengaged, and recordings were performed for an additional 60 minutes.

In Vivo/In Vitro Validation Studies

In order to further examine the relationship between real-time MMP activity and other biochemical assays, zymographic studies as well as in vitro MMP activity assays were used.

Gelatin Zymography

LV myocardial extracts (10 μg protein) were subjected to electrophoresis followed by gelatin (Novex 10% zymogram gel, 0.1% gelatin, Invitrogen) zymography.^{13,21,22} The size-fractionated MMP proteolytic regions were quantified by densitometry (Gel Pro Analyzer, Media Cybernetics).

In Vitro MMP Activity

In a subset of animals, tissue was collected at the termination of the protocol, was homogenized, and was subjected to in vitro MMP activity detection using the same MMP-1,2,3,7, and 9 fluorogenic substrate used in the in vivo real-time assay. Twenty micrograms of protein were loaded into a 96-well polystyrene plate (Nalge Nunc International, Rochester, NY) and were injected with 60 $\mu\text{mol/L}$ of MMP fluorogenic substrate. The reaction was allowed to proceed for 30 minutes at 37°C and fluorescence units were read on the FLUOstar Galaxy fluorescent microplate reader (280 and 360 nm, excitation and emission; BMG Labtechnologies). MMP-2/9 standards (1000 to 31.25 $\mu\text{g/mL}$; pretreated with APMA) were also incubated with the substrate to generate a standard curve.

Data Analysis

Hemodynamics and cardiac output were compared at each time point, and digitized sonomicrometric signals were analyzed to measure the distance between each crystal at end-systole and end-diastole and averaged more than 4 consecutive beats. These measurements were used to determine LV end-systolic and end-diastolic dimension and wall thickness, segmental shortening, and wall thickening. Peak circumferential wall stress was calculated as previously described using hemodynamic and sonomicrometry data.²³ Statistical analysis was performed using unpaired t-tests comparing each time point to baseline values and corrected for number of comparisons. Pair-wise comparisons were made between treatment groups at specific time points using paired t-tests. In addition, the 95% limit of agreement was determined using the Bland and Altman method for assessing agreement between 2 methods of clinical measurement.²⁴ All statistical analysis was done using the STATA statistical software package (Statacorp). Values of $P < 0.05$ were considered to be statistically significant.

Results

All of the pigs randomized to undergo AT₁RB or vehicle completed the intra-aortic balloon pump (IABP) protocol. IABP engagement caused a significant and sustained increase in LV peak pressure and aortic systolic pressure in both groups (Figure 1). After disengagement of the IABP, LV peak and aortic systolic pressure returned to near baseline levels. Summary hemodynamics are shown in Table 1. Despite significant increases in LV peak and aortic systolic pressure with IABP engagement, systemic hemodynamics remained relatively constant in both groups. Urine output fell dramatically in both the vehicle and AT₁RB groups whereas the IABP was activated but increased after IABP disengagement (156.7 ± 47.2 versus 34.9 ± 23.4 and 4.9 ± 3.2 mL, respectively). Plasma renin activity increased significantly with IABP engagement in both groups and was higher in the AT₁RB group consistent with pharmacological inhibition.

LV Segmental Shortening and Wall Stress Relationship

Segmental shortening fell significantly from baseline values in the vehicle group after IABP engagement and remained

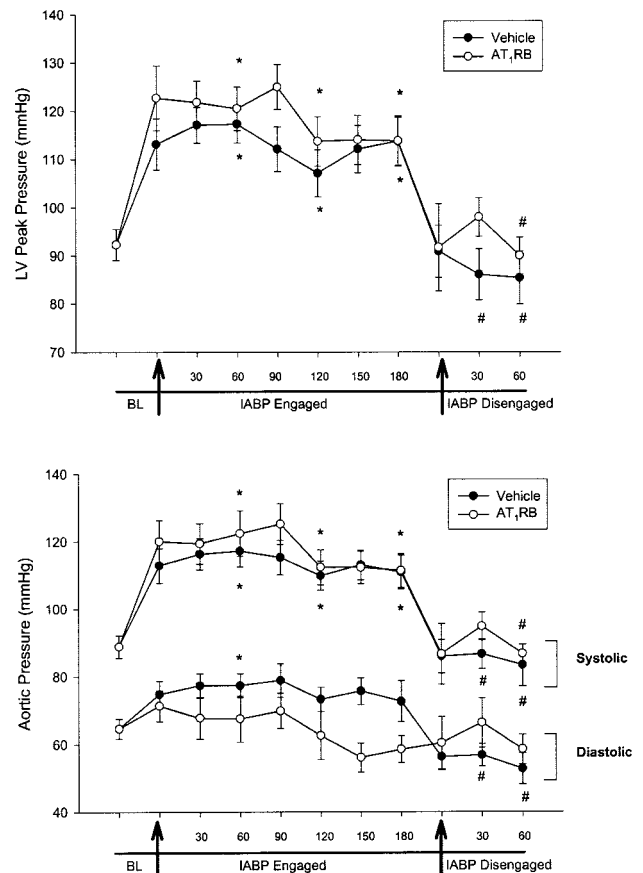


Figure 1. (Top) Left ventricular (LV) peak pressure significantly increased on engagement of the intra-aortic balloon pump (IABP) with or without angiotensin II type-1 receptor blockade (AT₁RB), and remained elevated until the IABP was disengaged. (Bottom) Aortic systolic blood pressure increased significantly in both treatment groups on IABP engagement; this increase was maintained for the duration of IABP engagement. Diastolic pressure was increased 1 hour after IABP engagement in the vehicle group. * $P < 0.05$ from Baseline; # $P < 0.05$ versus 180 minutes of IABP engagement in each group.

reduced after disengagement (Figure 2). Conversely, segmental shortening remained unchanged from baseline in the AT₁RB group throughout the protocol. In light of the fact that differential changes in segmental shortening occurred between groups, LV function was more carefully assessed using the LV stress-shortening relationship as described previously.^{20,25} Briefly, phenylephrine was infused incrementally into 3 additional normal pigs to titrate LV peak pressure from 80 to 200 mm Hg, and segmental shortening was measured across a range of isochronal peak wall stress values. A linear relationship was obtained (Figure 3), and isochronal stress-shortening values during IABP engagement from each treatment group were superimposed. Isochronal stress-shortening values in the vehicle and AT₁RB groups fell within the normal curve during IABP engagement. Stress-shortening in the AT₁RB group again remained within the normal stress-shortening values after IABP disengagement, but remained below the normal curve in the vehicle group.

Continuous MMP Activity Measurements

MMP activity fluorogenic measurements were taken at identical time points using a real-time flow-through fluorimetry

LV Function and Hemodynamic During and After Changes in LV Load

	Baseline	Aortic Balloon Pump ON (min)			Aortic Pump OFF (min)	
		60	120	180	30	60
Heart Rate, beats/minute	113.8 ± 5.4					
	<i>vehicle</i>	111.0 ± 7.8	117.8 ± 8.0	117.4 ± 6.3	125.8 ± 5.0	122.2 ± 4.8
	<i>AT₁RB</i>	121.5 ± 1.3	124.7 ± 2.7	124.7 ± 2.7	124.7 ± 2.7	125.3 ± 2.6
LV End-diastolic Pressure, mmHg	12.6 ± 1.8					
	<i>vehicle</i>	15.3 ± 3.0	15.5 ± 3.2	15.5 ± 2.4	11.9 ± 1.9	12.5 ± 1.8
	<i>AT₁RB</i>	14.2 ± 1.6	14.5 ± 1.7	13.5 ± 1.3	12.5 ± 1.5	12.9 ± 1.6
Mean Serial Pressure, mmHg	74.3 ± 3.2					
	<i>vehicle</i>	93.2 ± 4.2*	87.1 ± 4.2*	87.7 ± 6.2*	67.3 ± 6.0 [#]	70.0 ± 3.5 [#]
	<i>AT₁RB</i>	85.9 ± 6.5	79.2 ± 6.1	76.1 ± 3.9	67.9 ± 3.8	69.2 ± 7.7
Mean Pulmonary Artery Pressure, mmHg	28.3 ± 2.1					
	<i>vehicle</i>	26.6 ± 2.1	26.8 ± 2.4	26.4 ± 2.0	26.1 ± 2.2	24.9 ± 2.8
	<i>AT₁RB</i>	26.6 ± 1.4	29.3 ± 2.4	28.7 ± 3.3	29.9 ± 2.4	27.4 ± 2.5
Systemic Vascular Resistance, dynes*sec/cm ⁵	1712 ± 112					
	<i>vehicle</i>	1946 ± 126*	2006 ± 114*	2092 ± 138*	1613 ± 121 [#]	1686 ± 205
	<i>AT₁RB</i>	2500 ± 113*	2405 ± 167*	2409 ± 226*	2067 ± 127	1894 ± 163
Cardiac Output, L/min	3.29 ± 0.21					
	<i>vehicle</i>	3.17 ± 0.20	3.07 ± 0.27	3.12 ± 0.28	2.99 ± 0.23	2.94 ± 0.24
	<i>AT₁RB</i>	2.61 ± 0.28	2.54 ± 0.36	2.43 ± 0.27	2.71 ± 0.19	2.72 ± 0.23
LVED Diameter, mm	35.0 ± 1.3					
	<i>vehicle</i>	36.8 ± 1.5	36.7 ± 1.5	36.3 ± 1.6	34.4 ± 2.0	35.3 ± 2.0
	<i>AT₁RB</i>	33.9 ± 2.0	33.7 ± 1.9	33.1 ± 2.1	32.2 ± 1.9	32.0 ± 2.1
Wall Stress, g/cm ²	119.9 ± 9.4					
	<i>vehicle</i>	164.7 ± 24.3	146.2 ± 22.3	154.2 ± 20.8	108.3 ± 16.0	115.8 ± 19.9
	<i>AT₁RB</i>	149.5 ± 24.9	143.2 ± 26.5	133.0 ± 21.7	116.1 ± 23.6	108.2 ± 24.1
LVED Wall Thickness, mm	8.24 ± 0.55					
	<i>vehicle</i>	8.19 ± 0.63	8.33 ± 0.64	8.16 ± 0.62	8.60 ± 0.56	8.41 ± 0.61
	<i>AT₁RB</i>	8.19 ± 0.99	8.15 ± 1.03	8.34 ± 0.97	8.24 ± 1.05	8.23 ± 1.06
PRA, ng/mL/hr	3.19 ± 1.67					
	<i>vehicle</i>			13.70 ± 3.84		12.72 ± 4.41*
	<i>AT₁RB</i>			21.60 ± 4.02		12.47 ± 3.80

n=17 (baseline values), n=11 (vehicle values), n=6 (AT₁RB values), *P<0.05 vs baseline, #P<0.05 vs 180N of same group.

system as well as a fluorescence microplate reader. While the samples were collected from different microdialysis probes located within the same myocardial region, a high concordance between methods was observed (Figure 4A). In addition, the method by Bland and Altman for assessing agreement between 2 methods of clinical measurement²⁴ demonstrated that the data fell within the 95% limits of agreement (Figure 4B). Composite running averages of real-time interstitial MMP activity for both groups is shown in Figure 5. By 45 minutes of IABP engagement, MMP activity significantly decreased from baseline in both groups. On IABP disengagement, a surge in MMP activity occurred in the vehicle group, which was abrogated with AT₁RB. The rate of change in MMP activity after IABP disengagement was subjected to linear regression and revealed significantly higher MMP activity in the vehicle group as shown in Figure 5 (P=0.0431).

In Vivo/In Vitro Validation Studies

Real-time MMP activity was validated using 2 additional in vitro assays. Biopsies that were taken at baseline, after 180 minutes of IABP engagement, and at the end of the protocol, were subjected to gelatin zymography and an in vitro activity assay. There was a strong correlation between MMP-9 zymographic activity and real-time MMP activity (Figure 4C) and in vitro MMP activity on terminal myocardial samples and MMP activity during IABP disengagement (Figure 4D).

Discussion

Hypertension is the most common type of left ventricular (LV) pressure overload and a stimulus for LV remodeling and subsequent hypertrophy.⁴ LV pressure overload induces a number of biological and mechanical signals which result in myocyte growth and changes in the extracellular matrix (ECM).^{26,27} For example, neurohormonal stimuli such as

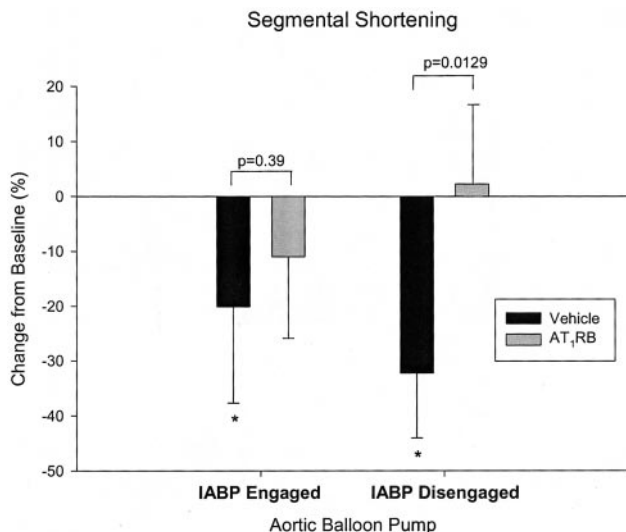


Figure 2. Segmental shortening significantly fell from baseline values in vehicle animals after intra-aortic balloon pump (IABP) engagement but remained statistically unchanged in animals treated with angiotensin II type-1 receptor blockade (AT₁R). With IABP disengagement, segmental shortening in the vehicle group was reduced but returned to baseline values in the AT₁R treated animals. A difference between vehicle and treated groups was demonstrated after IABP disengagement. * $P < 0.05$ versus baseline of 0.

release of angiotensin II (Ang II) and subsequent activation of the Ang II type I receptor (AT₁R) have been shown to contribute to LV remodeling with pressure overload.^{28,29} Whereas signals that drive myocyte growth have been a subject of intense study, determinants that cause ECM remodeling in LV pressure overload are not well understood. Mediators of ECM remodeling include the family of matrix metalloproteinases (MMPs).^{14,15} Accordingly, the goal of this study was to develop a novel strategy to directly measure interstitial MMP activity with the induction and termination of acute LV pressure overload as well as dissect out whether activation of the AT₁R contributes to this process. Using a large animal model in which LV pressure was controlled, the unique and significant findings of this study were 2-fold. First, real-time interstitial MMP activity was directly measured and changed in relation to LV load. Second, these changes in interstitial MMP activity were due, at least in part, to AT₁R activation. These results demonstrated that interstitial MMP activation is acutely responsive to changes in LV load, which in turn facilitates LV remodeling.

Whereas the present study is the first to directly measure myocardial interstitial real-time MMP activity, past clinical and experimental studies have shown that determinants of ECM synthesis and degradation are altered in response to LV pressure overload.^{10–12,30,31} Using surrogate markers of collagen synthesis/degradation and plasma MMP levels, clinical studies have demonstrated that collagen synthesis is upregulated with a decrease in plasma MMPs in untreated hypertensive patients.^{10,30} Several animal studies have demonstrated changes in myocardial MMP levels throughout the development of LV pressure overload hypertrophy.^{11,31} These studies, therefore, implied that MMP activity changes in response to load and alters ECM degradation; however,

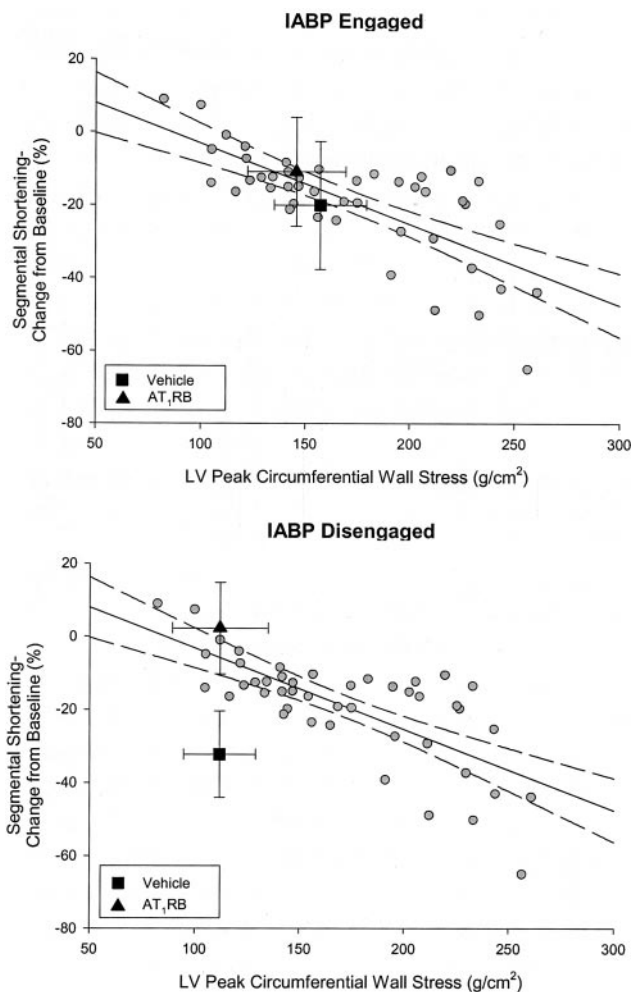


Figure 3. The LV stress-segmental shortening relationship was determined as described in the Results section. The stress-shortening relation was determined in normal pigs (gray circles), and this relation was linear ($r = 0.727$). The 95% confidence intervals are shown by dashed lines. (Top) With the intra-aortic balloon pump (IABP) engaged, the isochronal LV stress-shortening values remained within the stress-shortening relationship in the vehicle and AT₁R groups. (Bottom) Isochronal LV stress-shortening after IABP disengagement fell below the 95% normal confidence interval in vehicle group but remained within the stress-shortening relationship in the AT₁R group.

indirect or ex vivo assays that infer MMP activity can be problematic.^{10,12} Nevertheless, these past studies provided the impetus for the present study which developed a novel strategy to directly measure MMP activity during acute LV pressure overload. In this study, induction and termination of an acute LV pressure overload caused quantifiable changes in myocardial interstitial MMP activity. The results of the present study demonstrate the dynamic nature of the proteolytic system, which may not have been detected by ex vivo methods. Whereas the present study examined the changes in interstitial MMP activity with an acute LV pressure overload, whether and to what degree these changes persist with chronic alterations in LV load remains to be determined.

In order to examine whether interstitial MMP activity was influenced by the formation of Ang II and subsequent activation of the AT₁R, the present study administered an

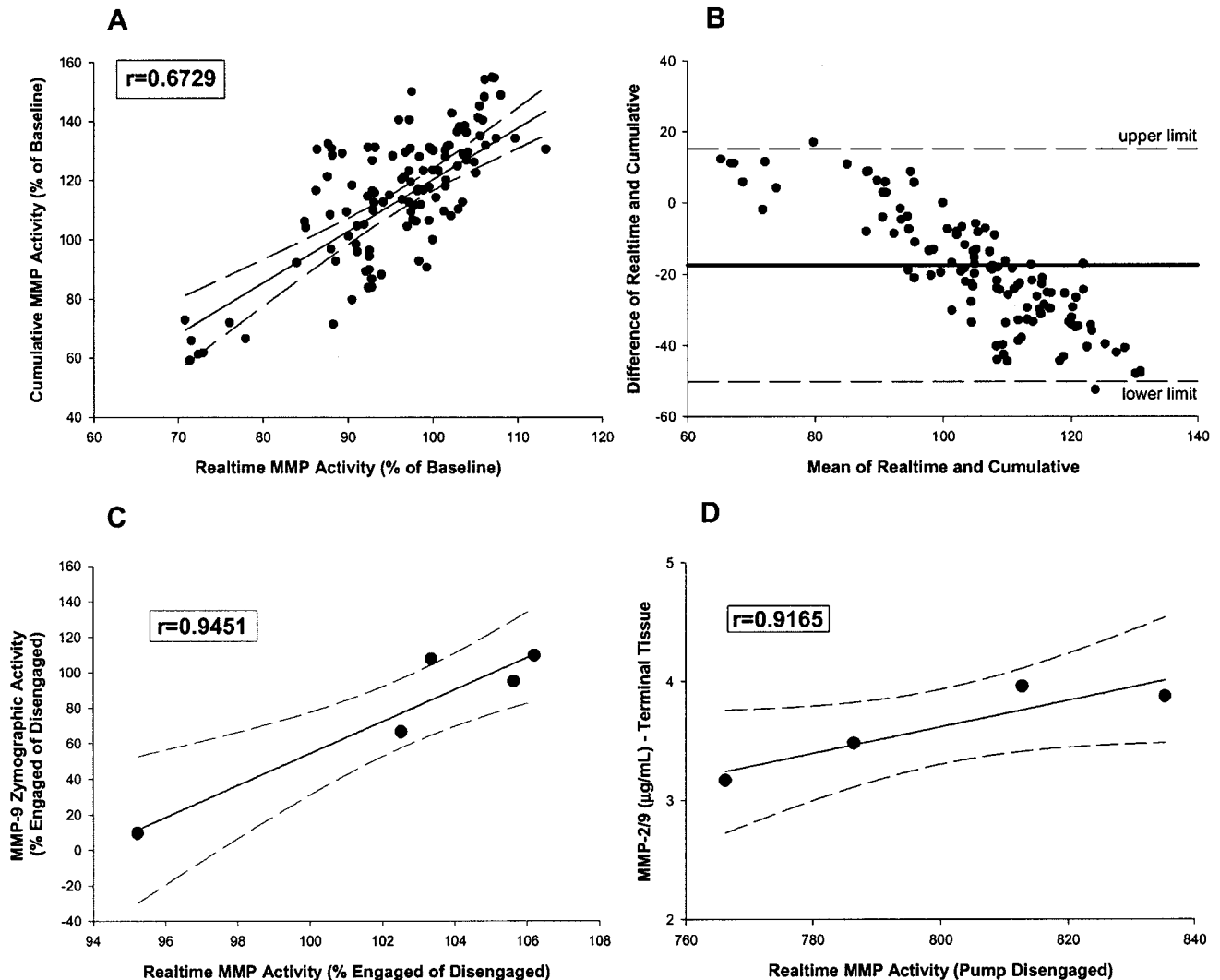


Figure 4. (A) MMP activity fluorogenic measurements were taken at identical time points using a real-time flow-through fluorimetry system as well as using a fluorescence microplate reader. While the samples were collected from different microdialysis probes located within the same myocardial region, a high concordance between methods was observed. (B) The Bland and Altman method was used to assess the agreement between 2 different methods of measurement. The upper and lower lines represent the 95% limits of agreement. (C and D) These graphs demonstrate a strong correlation between the real-time MMP activity measurement and *in vitro* measurements. Two separate analytical techniques, gelatin zymography (C) and *in vitro* MMP activity using tissue collected at the end of the protocol (D) revealed R values of 0.945 and 0.9165, respectively. (B, $n=5$; C, $n=4$).

AT₁R blocker (AT₁RB), which has been characterized in a porcine model previously.^{20,32} With AT₁RB, wall stress was unchanged, thus the AT₁R contributed to changes in LV geometry and myocardial performance after an induction of acute LV load. More importantly, however, after termination of acute LV load, the LV stress-shortening relationship was depressed in the vehicle group, while it remained within the normal range in the AT₁R inhibition group. It has been demonstrated previously that acute activation of MMPs causes changes in the myocyte-ECM interface which in turn will directly affect myocardial contractile performance.³³ Specifically, Baicu et al demonstrated that serine protease mediated activation of MMPs within myocardial papillary muscle preparations reduced isotonic shortening extent by $\approx 50\%$ when compared with untreated muscle preparations.³³

In the present study, termination of the LV afterload which was accompanied by a significant and persistent decline in LV myocardial performance (stress-shortening relation). One potential mechanism for this persistent decline in LV myocardial function is the surge in interstitial MMP activity, which occurred after termination of the LV afterload.

A cyclic variation in arterial pressure occurs in patients with the greatest increase in systolic blood pressure taking place in the morning hours.⁶ The present study demonstrated that during an acute induction of an increased oscillatory LV load, interstitial MMP activity declined. As discussed in a subsequent paragraph, this may have been the result of both neurohormonal as well as mechanical factors. Nevertheless, if the increased LV load was to persist, then it follows that the reduction in MMP activity would result in reduced ECM

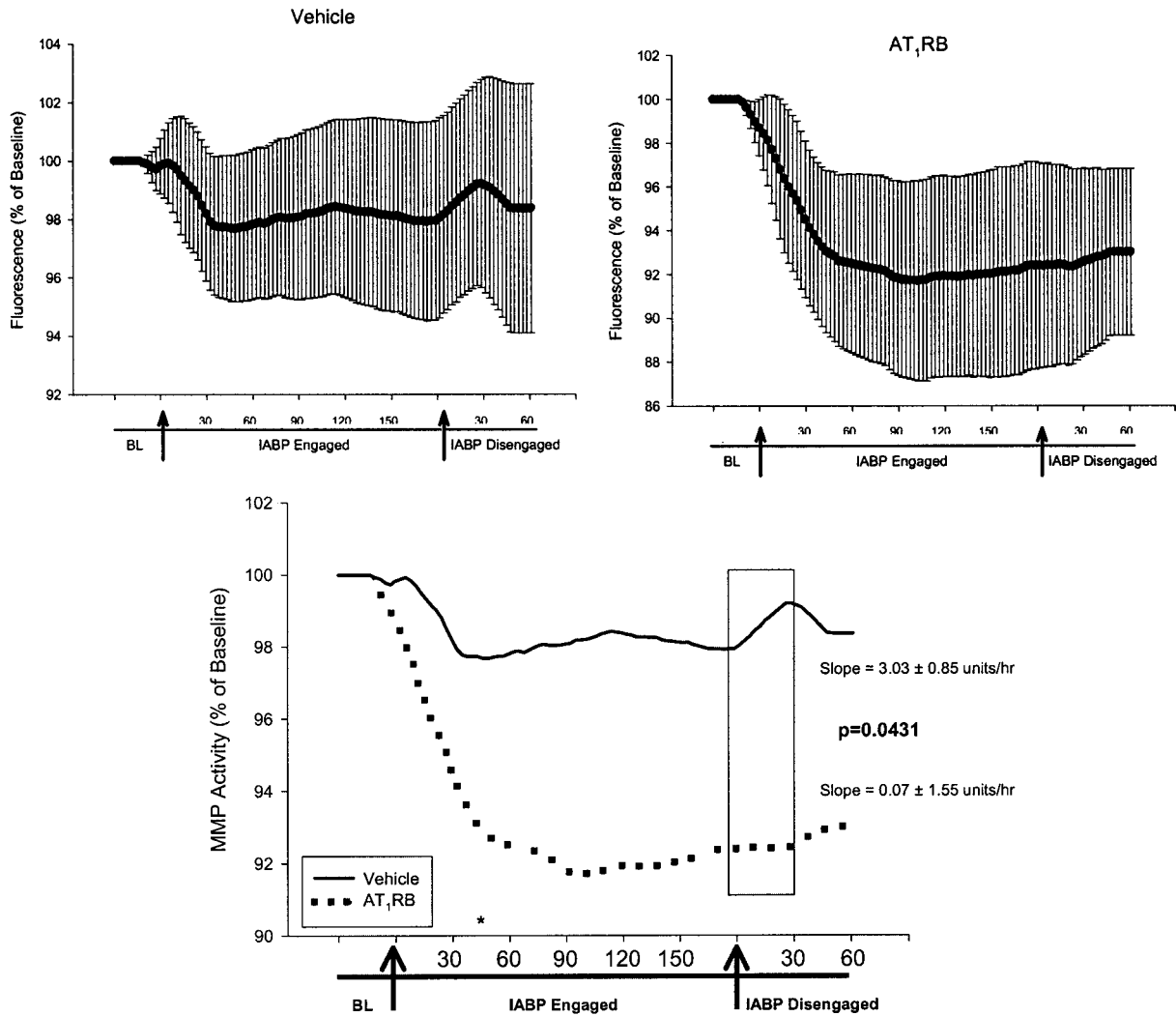


Figure 5. (Top) Composite graphs of real-time MMP activity with error bars in both vehicle animals (left) and animals treated with angiotensin II type-1 receptor blockade (AT₁RB) (right). Fluorescence is plotted as a percent change from baseline values. MMP activity curves were smoothed using a running average method. (Bottom) On intra-aortic balloon pump (IABP) disengagement, a surge in MMP activity occurred in the vehicle group that was abrogated with AT₁RB. Specifically, the rate of change in MMP activity after IABP disengagement was subjected to linear regression. Significantly higher MMP activity occurred during this time interval in the vehicle group ($P=0.0431$). The time period used to compute the rate of change in MMP activity has been expanded in the figure. * $P<0.05$ at 45 minutes versus baseline.

turnover and fibrillar collagen accumulation, a histological hallmark of pressure overload hypertrophy.¹⁻³

Hormonal systems, growth factors, and cytokines are all activated in response to LV pressure overload.^{34,35} Among these factors, the effects of Ang II have been extensively studied.^{8,13} Beyond its pressor effects, Ang II has been shown to modulate collagen synthesis and MMP/TIMP expression.^{9,13} On AT₁R activation, isolated cardiac myocytes have been shown to significantly increase MMP-2, MMP-9 and MT1-MMP (MMP-14) abundance.¹³ In addition, an AT₁R antagonist has been shown to reduce the upregulation of MMP-1 mRNA and expression in a rabbit model of hypercholesterolemia.³⁶ A potential mechanism for the further reduction in MMP activity in the AT₁RB group may be caused by the decreased MMP transcription or preserved levels of TIMPs. In a parallel set of studies, the present

investigation demonstrated that AT₁RB abrogated the surge in MMP interstitial activity and was associated with a normalization of LV myocardial function. It is likely that the effects of AT₁RB on interstitial MMP activity were caused by a combination of mechanical (LV wall stress) as well as biological signaling.

In the present study, the substrate detected activity for a wide spectrum of MMP types (MMP-1, 2, 3, 7, and 9). Therefore, differentiating which MMP types contributed to the changes in MMP activity during induction and termination of acute LV overload could not be determined. A previous study conducted by this laboratory used a substrate specific for membrane type-1 MMP to investigate changes in activity during ischemia and reperfusion.¹⁷ Further studies that use more specific MMP substrates to measure real-time interstitial MMP activity during changes in LV load are

warranted. Past studies have clearly demonstrated that an increase in mechanical LV load caused contractile protein synthesis and indicated a rapid myocyte response.³⁷ Using the real-time microdialysis technique, a rapid adaptive change in MMP activity was observed in response to alterations in load. Whereas the translation of the findings from the present acute study of LV afterload to that of LV hypertrophy must be done with extreme caution, 2 recent correlative studies can be integrated with the present findings. First, in patients with hypertension and developing LVH, there appears to be higher levels of TIMPs which would yield a reduction in myocardial MMP activity^{22,23} - a directional change which was observed in the present study with induction of an acute pressure overload. Second, Sakata and colleagues reported that relative changes in myocardial MMP levels preceded changes in LV geometry and function in hypertensive rats.³⁸ Thus, the changes in interstitial MMP activity that occur as a function of LV load may be an early biochemical event in the maladaptive process which occurs with LV hypertrophy and remodeling. Whereas traditionally considered a static structure, the ECM and its rate of turnover, mediated through this proteolytic system, appear to be modified immediately on induction and termination of increased LV load. Identifying signaling and transduction pathways that contribute to MMP activation may yield therapeutic targets to modify ECM structure and function with LV hypertrophy.

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