Endothelin-1 mediates cardiac mast cell degranulation, matrix metalloproteinase activation, and myocardial remodeling in rats

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Endothelin-1 mediates cardiac mast cell degranulation, matrix metalloproteinase activation, and myocardial remodeling in rats. The objective of this study was to determine whether ET-1 is capable of stimulating cardiac mast cell degranulation, matrix metalloproteinase (MMP) activation, and myocardial remodeling in rats. Am J Physiol Heart Circ Physiol 287: H2295–H2299, 2004. First published July 1, 2004; doi:10.1152/ajpheart.00048.2004.—The objective of this study was to determine whether ET-1 is capable of stimulating cardiac mast cell degranulation, matrix metalloproteinase (MMP) activation, and myocardial remodeling in rats.

CHRONIC VENTRICULAR VOLUME OVERLOAD induces characteristic myocardial remodeling (i.e., ventricular wall thinning and chamber dilatation) in part through transient degradation of the extracellular matrix (ECM) (5, 6, 16, 17). The role of matrix metalloproteinases (MMPs) in the degradation of the ECM has been extensively studied (4, 9, 12, 15, 31), and although recent findings from our laboratory indicate that cardiac mast cells play a central role in activating MMPs (4, 9), the factor(s) responsible for initiating mast cell-mediated MMP activation has yet to be identified. Mounting evidence has strongly correlated elevated circulating and tissue levels of endothelin (ET)-1 with the severity of heart failure in patients and animal models (20, 21, 28). In addition, treatment with ET-1 receptor antagonists has been shown to prevent MMP activation and attenuate ventricular dilatation in animal models of heart failure (13, 14, 30). Accordingly, the goal of this study was to determine whether ET-1 is capable of stimulating cardiac mast cell degranulation, which in turn causes MMP activation and degradation of the ECM. Consistent with this hypothesis, ET-1 has been shown to cause degranulation in noncardiac mast cells (3, 35), and a study by Brown et al. (7) using the aortocaval (AV) fistula model of chronic volume overload demonstrated increased cardiac mRNA expression of the ET system, specifically, ET-1 and the ETA receptor subtype. To this end, ET-1 was infused in blood-perfused isolated rat hearts to evaluate its effects on cardiac mast cells. Administration of ET-1 induced extensive cardiac mast cell degranulation, myocardial edema, MMP activation, ECM degradation, and left ventricular (LV) dilatation.

MATERIALS AND METHODS

All experiments were performed using adult male Sprague-Dawley rats housed under standard environmental conditions and maintained on commercial rat chow and tap water ad libitum. All studies conformed to the principles of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by our institution’s Animal Care and Use Committee. Anesthesia for the experimental procedure was affected by pentobarbital sodium (50 mg/kg ip).

Experimental design. The experimental groups included an untreated control group exposed to saline (n = 5), an untreated group perfused with ET-1 (n = 9), and a nedocromil sodium-treated group perfused with ET-1 (n = 6). Nedocromil sodium was administered via a 21-day time-release pellet (Innovative Research; Sarasota, FL) implanted subcutaneously 5 days before isolation of the heart to achieve a dosage of 30 mg·kg⁻¹·day⁻¹. Preliminary studies confirmed that administration of 30 mg·kg⁻¹·day⁻¹ nedocromil sodium effectively prevented both the increase in mast cell density and MMP activation at 24 h in the AV fistula model (data not shown). The experiments were performed using an isolated, blood-perfused heart preparation as previously described (5). Briefly, the ascending thoracic aorta in the anesthetized rat was cannulated for continuous retrograde perfusion of the heart via an apparatus consisting of a pressurized perfusion reservoir (100–105 mmHg) and a collection reservoir connected in circuit with a support rat. The extirpated heart was attached to the apparatus for perfusion with oxygenated blood obtained from the support rat via a carotid artery catheter. The coronary venous effluent was collected in a reservoir and returned to the support rat via a jugular vein catheter. The support rat and blood donors utilized for studying the nedocromil-treated group were also pretreated with nedocromil in an identical fashion to the experimental animals. After removal of the left atrium, a highly compliant latex balloon was inserted into the ventricular chamber to obtain LV pressure-volume (P-V) relationships. The proximal end of the balloon was connected via a short piece of tubing to a three-way stopcock that was used to adjust the balloon volume through one port while measuring LV pressure using a pressure transducer (Transpac IV, Abbott Critical Care Systems; North Chicago, IL) attached to the remaining port. Once the heart developed stable isovolumetric con
tractions, the balloon volume that produced an LV end-diastolic pressure (LVEDP) of 0 mmHg was used to determine baseline ventricular volume (V₀). The volume in the balloon was then increased in 5- to 10-μl increments until an LVEDP of 25 mmHg was attained. The end-diastolic and peak isovolumetric pressures were recorded after each increase in balloon volume, with three to four data sets collected to ensure that the preparation was stable.

To assess the effect of ET-1 on coronary flow and mast cell degranulation, 1 ml of blood containing ET-1 (Sigma; St. Louis, MO) was administered to the isolated heart of normal or nedocromil-treated rats by its introduction into the pressurized perfusion reservoir, allowing the solution to mix with the reservoir blood to achieve a final ET-1 concentration of 20 pg/ml. This pathophysiological dosage was selected based on 1) preliminary observations of tissue ET-1 levels in the infrarenal AV fistula model of chronic volume overload (data not shown), 2) baseline plasma ET-1 levels of ~9.5 pg/ml reported in previously published studies using Sprague-Dawley rats, and 3) an expected two- to threefold increase in ET-1 characteristic of heart failure regardless of etiology (8, 18, 23). Coronary venous effluent was collected for 3 min immediately before and after ET-1 administration to determine coronary flow. The coronary venous blood containing ET-1 was not returned to the support rat. P-V relationships were obtained for each heart before and 30 min after infusion of ET-1. Control hearts underwent the same blood-perfused isolation procedure and were obtained for each heart before and 30 min after infusion of ET-1. Each gel was run in duplicate, and, to compare results from different gels, a single extract from the same control heart was used as a standard on all gels. The activities of the lytic bands in the other lanes of an individual gel were expressed as a percentage of this standard’s activity. The MMP activity values for each sample were normalized for their protein concentration using a Bio-Rad protein assay. Once normalized in this fashion, the percentage of MMP-2 and MMP-9 activity from control and ET-1-perfused isolated heart groups were averaged.

Statistical analysis. Statistical analyses were performed with GraphPad Prism 4.0 (San Diego, CA). All grouped data are expressed as means ± SD except where noted. Grouped data comparisons were made by one-way ANOVA with intergroup comparisons analyzed using Bonferroni post hoc testing. Statistical significance was taken to be P < 0.05. For each heart, diastolic P-V relationships were fit to a third-order nonlinear regression (GraphPad Prism 4.0). The volumes corresponding to the pressure values at 2.5-mmHg increments between 0 and 25 mmHg were determined and corrected for the volume displaced by the balloon (i.e., mass of the balloon in the ventricle divided by the density of the latex). For each heart and for each EDP, the corresponding volumes were then averaged to obtain the final P-V relationship. P-V curves were analyzed using a two-factor repeated-measures ANOVA.

RESULTS
Evidence of extensive mast cell degranulation in isolated hearts treated with ET-1 was found on histological examination of all treated hearts (Fig. 1). Additionally, the percentage of mast cell degranulation was determined based on ×400 microscopic examination of the entire LV section of four randomly selected hearts from control and treated groups. Degranulation was defined as the presence of disseminated granules external to the cell membrane and/or loss of membrane integrity. The number of mast cells that were degranulated was...
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Fig. 2. A: representative zymogram depicting matrix metalloproteinase (MMP)-2 activity in control (C) and ET-1-treated isolated hearts as well as normal tissue extract (NormTissue) and tissue extract incubated with ET-1 (NormTissue+ET-1). B: comparison of average active MMP-2 levels in control (expressed as 100%) and ET-1-treated isolated hearts and ET-1 plus nedocromil sodium (Nedoc)-treated and ET-1-treated tissue extracts from normal hearts. Values are means ± SD. *P < 0.01 vs. control.

 sigificantly higher in the ET-1-treated hearts compared with control and nedocromil-treated hearts (70 ± 3.5% vs. 25 ± 5.0% and 37 ± 8.5%, respectively, P < 0.05). Concomitant with cardiac mast cell degranulation, there was a significant increase in myocardial %H2O in the ET-1-treated hearts relative to control hearts (78.8 ± 1.5% vs. 74.2 ± 2.2%, respectively, P < 0.01). Development of myocardial edema was prevented in the nedocromil-treated hearts. Average coronary flow was not significantly different in ET-1-treated isolated hearts as measured by timed collection of coronary effluent immediately before and after ET-1 administration (2.57 vs. 2.21 ml/min, respectively).

Figure 2A illustrates a representative zymogram depicting MMP-2 activity in control and endothelin-1-treated isolated hearts as well as normal tissue extract and tissue extract incubated with ET-1. The average percent change in MMP-2 activity obtained in control, ET-1-treated, and ET-1 + nedocromil-treated hearts is summarized in Fig. 2B. ET-1 infusion resulted in a 107% increase in MMP-2 activity above that in control hearts, whereas pretreatment with nedocromil completely blocked ET-1-mediated MMP-2 activation. To determine whether this MMP-2 activation was a direct or indirect effect of ET-1, cardiac tissue extracts from four normal hearts were incubated for 30 min before being analyzed in duplicate by zymography with the same concentration of ET-1 (20 pg/ml) that was administered in the isolated heart studies. There was no significant difference in MMP-2 activity between control and samples incubated with ET-1 in the in vitro study, indicating that ET-1 does not act directly to activate MMPs (Fig. 2, A and B). Rather, these results indicate that the ET-1-induced increase in MMP-2 activation was due to ET-1-mediated mast cell degranulation. Although MMP-9 bands were present in all groups, no significant difference in MMP-9 activity was observed between control, ET-1-treated, and ET-1 + nedocromil-treated hearts.

CVF was measured to determine whether ET-1-induced MMP activation resulted in degradation of the ECM. A marked reduction in CVF in ET-1-treated hearts relative to control was obtained (0.69 ± 0.09% vs. 0.99 ± 0.04%, respectively, P = 0.001), whereas inhibition of mast cell degranulation with nedocromil pretreatment maintained CVF near control values (0.84 ± 0.03% vs. 0.99 ± 0.04%, respectively, P = 0.45).

The average LVEDP and LV end-diastolic volume (LVEDV) relationships before and after ET-1 administration are depicted in Fig. 3. In ET-1-treated hearts, LVEDV at an LVEDP pressure of 0 mmHg (V0) was increased from 298.9 ± 17.4 to 330.2 ± 22.1 µl (P = 0.07) after ET-1 administration, reflecting a strong trend approaching significance for development of ventricular dilatation. However, myocardial compliance as assessed by the volume required to increase LVEDP from 0 to 25 mmHg (ΔV25) was not different after ET-1 administration (77.3 ± 13.8 vs. 82.4 ± 17.1 µl, P > 0.6). These data reflect a parallel shift to the right, consistent with moderate ventricular dilatation, despite the presence of significant myocardial edema after exposure to ET-1. This rightward shift after ET-1 administration was observed in seven of nine hearts. The two hearts that did not develop this rightward shift despite extensive mast cell degranulation and elevated MMP-2 activity had the highest (81.7%) %H2O values. Similarly, the V0 shift for the heart with the second highest value (79.7%) %H2O was minimal (~6 µl).

Furthermore, linear regression analysis of %H2O versus the change in V0 pre- and post-ET-1 administration from all nine ET-1-treated hearts was inversely correlated (r2 = 0.76), indicating that the greater the degree of edema present, the less extensive the degree of ventricular dilatation. Administration of nedocromil initiated 5 days before the isolated heart study completely abrogated the ET-1-induced increase in V0.

The slopes of the peak isovolumic pressure (Pmax)-LVEDV relationship has been shown to be an accurate indicator of LV contractility (33). Assessment of the slopes of the Pmax-LVEDV relationship established that contractility in the ET-
1-treated hearts was not significantly different from control (0.992 ± 0.30 vs. 0.962 ± 0.27 mmHg/µl, respectively, P > 0.74).

**DISCUSSION**

Although increased MMP activity has been observed in several models of heart failure (i.e., secondary to rapid pacing, myocardial infarction, and chronic volume overload) (6, 15, 29, 31, 32), very little is known with regard to the factor(s) responsible for MMP activation. Our recent findings implicate cardiac mast cell degranulation as an in vivo mechanism responsible for activating MMPs and the subsequent ECM degradation (4, 6, 9, 10, 32); however, the stimulus initiating these cardiac mast cell-mediated effects has not been elucidated. Specifically, the intrinsic events and/or hormonal agents that initiate cardiac mast cell degranulation are not known. In this study, we focused on the neurohormone ET-1 because the elevation in plasma and ventricular tissue levels of ET-1 corresponds to the severity of heart failure in both human patients and animal models (20, 21, 23, 28).

Several studies have established MMP activation as causing the ventricular dilatation characteristic of the failing heart (4, 10, 31). Typically, increased MMP activity causes a rapid disruption of the ECM as measured by a reduction in CVF or hydroxyproline (19, 22, 26, 27, 31). Other studies have shown that treatment with ET receptor antagonists significantly attenuates ventricular remodeling during the progression of congestive heart failure in rats (13, 14, 22, 25, 27); however, the potential mechanisms mediating these phenomena were thought to lie in the reduction of afterload, thereby lessening LV wall stress and limiting the stimulus for progressive ventricular remodeling. Accordingly, this is the first study to establish a specific mechanism mediating the structural remodeling induced by pathophysiological elevations in ET-1. This study demonstrated that ET-1-induced mast cell degranulation produced a marked increase in the levels of active MMP-2. Conversely, incubation of normal myocardial tissue extracts with ET-1 at the same dose and for the same duration as that used in the isolated heart preparation did not produce an increase in MMP activity. Moreover, prevention of MMP-2 activation by treatment with the mast cell stabilizer nedocromil sodium firmly establishes that ET-1-induced MMP-2 activation in the heart is mediated via degranulation of resident cardiac mast cells. This novel finding that ET-1 increases MMP activity indirectly through the activation of cardiac mast cells suggests that ET-1 receptor antagonism might be effective in preventing myocardial remodeling. Indeed, that hypothesis is supported by other studies that have shown that ET-1 receptor antagonism attenuates the myocardial remodeling process by inhibiting MMP activation (3, 13, 14, 30). Specifically, Podesser et al. (30) reported that ETA receptor subtype blockade by sitaxsentan prevented MMP activation after myocardial infarction in rats. Although others have found that noncardiac mast cells are responsive to ET-1 (3, 34, 35), the findings in this study are the first to demonstrate that ET-1 is capable of causing cardiac mast cell degranulation, thereby activating MMPs, which in turn produce ECM degradation. Thus the findings of others combined with the results herein indicate that ET receptor-mediated stimulation of mast cell degranulation is responsible for ET-1-induced MMP activation.

Significant myocardial edema was observed as a result of ET-1-induced mast cell degranulation. Although histamine release was not measured in these hearts after ET-1 administration, Chancy et al. (9) found comparable increases in myocardial edema largely due to increased vasopermeability produced by documented histamine release from cardiac mast cells exposed to compound 48/80. It should also be noted that several previous studies have demonstrated that comparable increases in %H2O typically resulted in a nonparallel leftward shift in the LV diastolic P-V relationship. For example, Cross et al. (11) found that decreased distensibility in canine hearts was proportional to the extent of myocardial interstitial fluid accumulation. Similarly, Amirhamezeh et al. (1) found that a 4.5% increase in myocardial water content resulted in a nonparallel leftward shift in the P-V relationship in rat hearts. Accordingly, the parallel rightward shift in the P-V relationship obtained herein indicates that the significant MMP activation and subsequent interstitial collagen degradation produced ventricular dilatation and increased compliance, which offset the expected edema-related increase in myocardial stiffness. An alternative possibility of compromised coronary perfusion contributing to or enhancing the rightward shift in the P-V relationship of these hearts is refuted by the observation that coronary flow was unchanged and systolic function was preserved.

Previous findings from our laboratory have demonstrated that a 30-min perfusion of compound 48/80 induced 1) mast cell degranulation; 2) significant activation of myocardial MMPs; 3) a moderate rightward shift in the P-V relationship (i.e., an increase of 32 µl in EDV at an EDP of 0 mmHg), the extent of which was lessened by significant myocardial edema; and 4) a 52% reduction in CVF (9). In that study, it was concluded that degradation of the interstitial collagen matrix was sufficient to counteract the expected decrease in diastolic compliance due to the significant increase in myocardial water content. Similar observations were made by MacKenna et al. (19) utilizing bacterial collagenase-perfused rat hearts. That is, they observed a significant increase in V₀ of ~55 µl that was associated with a 36% decrease in CVF. Although the rightward shift during the 60-min collagenase perfusion was progressive, the largest shift occurred within the first 30 min. However, in that study, the collagenase infusion did not result in an increase in myocardial %H2O above that in the control group. Consequently, although there was a trend for the non-collagenase-perfused P-V curves to rotate to the left (i.e., become stiffer) over the 1-h study period, the ECM degradation produced a rightward shift in the P-V relationship of the collagenase-perfused hearts that was essentially parallel. In the present study, we observed analogous results with ET-1-mediated mast cell degranulation producing marked MMP activation and extensive interstitial collagen degradation, both of which were prevented by pretreatment with nedocromil sodium.

In summary, this is the first study to demonstrate ET-1-induced degranulation of cardiac mast cells. This is also the first study to conclusively demonstrate that MMP activation is not a direct effect of ET-1 but rather involves ET-1 dependent degranulation of cardiac mast cells. Consequently, we conclude that ET-1 is capable of inducing cardiac mast cell degranulation, thereby producing the subsequent increase in MMP activity, ECM degradation, and ventricular dilatation.
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GRANTS

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