Cardiac interstitial bradykinin and mast cells modulate pattern of LV remodeling in volume overload in rats

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Wei, Chih-Chang, Pamela A. Lucchesi, Jose Tallaj, Wayne E. Bradley, Pamela C. Powell, and Louis J. Dell'Italia. Cardiac interstitial bradykinin and mast cells modulate pattern of LV remodeling in volume overload in rats. Am J Physiol Heart Circ Physiol 285:H784–H792, 2003. First published March 27, 2003; 10.1152/ajpheart.00793.2001.—In the current study, interstitial fluid (ISF), bradykinin (BK), and angiotensin II (ANG II) levels were measured using cardiac microdialysis in conscious, nonsedated rats at baseline and at 48 h and 5 days after each of the following: sham surgery (sham, n = 6), sham + administration of ANG-converting enzyme inhibitor ramipril (R, n = 6), creation of aortocaval fistula (ACF, n = 6), ACF + R (n = 6), and ACF + R + BK2 receptor antagonist (HOE-140) administration (n = 6). At 5 days, both ISF ANG II and BK increased in ACF rats (P < 0.05); however, in ACF + R rats, ISF ANG II did not differ from basal levels and ISF BK increased greater than threefold above baseline at 2 and 5 days (P < 0.05). Five days after ACF, the left ventricular (LV) weight-to-body weight ratio increased 30% (P < 0.05) in ACF but did not differ from sham in ACF + R and ACF + R + HOE-140 rats despite similar systemic arterial pressures across all ACF groups. However, ACF + R + HOE-140 rats had greater postmortem wall thickness-to-diameter ratio and smaller cross-sectional diameter compared with ACF + R rats. There was a significant increase in mast cell density in ACF and ACF + R rats that decreased below sham in ACF + R + HOE-140 rats. These results suggest a potentially important interaction of mast cells and BK in the cardiac interstitium that modulates the pattern of LV remodeling in the acute phase of volume overload.

hypertrophy; chymase; angiotensin-converting enzyme

TREATMENT with angiotensin-converting enzyme (ACE) inhibitors has been shown to improve survival in patients with advanced heart failure as well as those who receive it after myocardial infarction (7, 31, 34). It is hypothesized that ACE inhibitors exert beneficial effects by inhibiting cardiac tissue ACE and ANG II production thus attenuating unfavorable remodeling of the left ventricle (LV). In addition to generating ANG II from ANG I, ACE also catalyzes the degradation of bradykinin (BK) to inactive metabolites with more favorable kinetics for hydrolysis of BK than for conversion of ANG I to ANG II (21, 42). The concept that BK may play a more prominent role in the mechanism of action of ACE inhibitors has gained further credence due to the identification of alternative ANG II-forming pathways that bypass ACE (12). In particular, the serine protease heart chymase has been identified in multiple organs of all mammals (37) and its catalytic efficiency for the hydrolytic conversion of ANG I to ANG II is substantially higher than that of ACE and other ANG II-forming enzymes (9, 38).

In a comparison of ANG II formation in heart tissue extracts from humans and animals, we found that >90% of ANG II formation was from chymase in human and dog hearts, whereas >80% of ANG II formation was from ACE in rat, rabbit, and mouse hearts (1). However, there is mounting evidence that increased chymase expression plays a role in ANG II-dependent control of blood pressure and cardiac hypertrophy in mice and rats. Chymase has been implicated in the development of hypertension in spontaneously hypertensive rats (15). ANG II formation from chymase is increased in hearts of mice with volume-overload hypertrophy (30). Conditional overexpression of a rat ANG II-forming chymase in mouse blood vessels leads to hypertensive arteriopathy (16). In the human heart and its vessels, chymase is chiefly synthesized in mast cells and is present in the cardiac interstitium (37). Increased numbers of cardiac mast cells have been reported in human patients with end-stage cardiomyopathy (29) as well as in rat models of hypertension (28), myocardial infarction (13), and volume overload secondary to an aortocaval fistula (ACF; Ref. 3). Although the source of mast cell influx is unknown, the cells accumulate in a relatively short period of time (i.e., 12 h) in the ACF rat model, which indicates that these additional mast cells may be an important source of chymase in volume overload (3).

We hypothesized that the effects of ACE-inhibitor treatment in the early phase of cardiac volume overload due to ACF in rats are determined largely by augmenting BK rather than reducing ANG II due to increased mast cell density and chymase expression. To test this hypothesis, microdialysis probes were implanted into myocardia of rats and were used to collect
interstitial fluid (ISF) to measure ISF ANG II and BK from the myocardia of fully conscious rats before and after induction of ACF.

METHODS

Animal Preparation

Adult Sprague-Dawley rats underwent pentobarbital sodium anesthesia and mechanical ventilation with a Harvard ventilator. Insertion of the microdialysis probe into the heart was performed as previously described (5). An incision was made between the sixth and seventh ribs on the left side, intercostal muscles were cut, and the ribs were spread. The microdialysis probe was inserted caudal to rostral in the left ventricular (LV) myocardium. To fix the cannula, a stitch was made near the area where the cannula leaves the heart. Lungs were inflated, and the sixth and seventh ribs were sutured together. The tubing of the probe exited the thoracic cavity one intercostal space above and below the incision. The ends of the probe were transferred subcutaneously to the base of the neck and exteriorized, plugged, and secured with dental acrylic thread. When all surgery was complete, a 25-gauge needle was inserted into the thoracic cavity to withdraw any remaining air and fluid from the thoracic cavity. Animals were not returned to their housing area until they were awake and able to stand. Subsequent experiments were carried out when animals were fully recovered after 24 h. This animal protocol was approved by the Animal Resource Program at the University of Alabama at Birmingham.

Surgical Preparation for ACF

Twenty-four hours after insertion of the microdialysis probe in the heart, an infrarenal abdominal aorta-to-vena cava fistula was created in the rats as was previously performed in our laboratory (35). Briefly, a ventral abdominal laparotomy was performed to expose the aorta and caudal vena cava ~1.5 cm below the renal arteries. Via blunt dissection, the overlying adventitia was removed and the vessels were exposed; care was taken not to disrupt the tissue that connected the vessels. Both vessels were then occluded proximal and distal to the intended puncture site, and an 18-gauge needle was inserted into the exposed abdominal aorta and advanced through the medial wall into the vena cava to create the fistula. The needle was withdrawn, and the ventral aortic puncture was sealed with cyanoacrylate. Creation of the ACF was visualized by the pulsatile flow of oxygenated blood into the vena cava. The abdominal musculature and skin incisions were closed by standard techniques with absorbable suture and autoclips.

Thirty 10-wk-old Sprague-Dawley rats with indwelling cardiac microdialysis probes had ISF collections while in the conscious, nonsedated condition at baseline, at 48 h, and at 5 days after each of the following: sham surgery (sham, n = 6), sham + ACE inhibitor ramipril administration (sham + R, n = 6), ACF (n = 6), ACF + R (n = 6), and ACF + BK₂-receptor blockade (with HOE-140; n = 6). Oral administration with ACE inhibitor (ramipril, 10 mg·kg⁻¹·day⁻¹; Hoechst; Frankfurt, Germany) was started after induction of ACF with or without BK₂ receptor blockade (HOE-140, 500 μg·kg⁻¹·day⁻¹; American Peptide; Sunnyvale, CA; Refs. 19 and 40). HOE-140 was injected subcutaneously daily.

At the time of death, aortic pressure, LV peak systolic and LV end-diastolic pressures, and peak ±dP/dt were measured in anesthetized animals using an intravascular pressure transducer (model SPR-249A, Mikro-Tip catheter transducer; Millar Instruments; Houston, TX) introduced via the right carotid artery into the aorta. After death, the LV, right ventricle (RV), and the lungs were weighed, collected, snap-frozen in liquid nitrogen, and stored at −80°C.

Cardiac Microdialysis Technique in Rats in Vivo

The cardiac microdialysis technique used in this study was similar to that employed in our previous studies using dogs (11, 39) and rats (5). Each microdialysis probe (Clirans, Terumo; Tokyo, Japan) is a semipermeable membrane with a molecular mass cutoff of 35 kDa and an inner diameter of 200 μm, which is connected to methyl-deactivated silica capillary tubing (OD, 0.17 mm) and inserted to polyethylene (PE)-10 tubing. Each microdialysis probe consists of a single 200-μm dialysis fiber and two hollow tubes inserted, adjusted, and sealed within the dialysis fiber such that the distance between the ends of the silica tubes is 2.2 mm. The probe was perfused by a precision infusion syringe pump (BAS; West Lafayette, IN) at a flow rate of 1.0 μl/min. In each animal, one sterilized microdialysis probe was implanted into the left ventricular myocardium. After microdialysis perfusate (in vitro) experiments, the inflow capillary tube of each was connected via PE-10 tubing to a PE-50 tube using a syringe filled with lactated Ringer solution and was perfused at 1.0 μl/min. The effluent, or dialysate, was collected from the outflow silica tube in small plastic tubes with 10 μl of acetic acid (2.5 M) for ANG peptides and with 98% ethanol for BK and was frozen (at −80°C) until biochemical analysis.

Cardiac microdialysis is based on the principle that as the dialysate solution passes through the microdialysis fiber, diffusion occurs between the fluid within the fiber and the ISF surrounding the fiber. The dialysate concentration is therefore an estimate of the intramyocardial ISF concentration. However, at the flow rates that are used in microdialysis experiments in vivo, it is unlikely that complete equilibration occurs between the lactated Ringer solution within the fiber and the cardiac ISF in the vicinity of the fiber. Therefore, we performed in vitro experiments to estimate recovery from microdialysis probes as we previously described (5, 11, 39).

With the assumption that all probes have the same area available for diffusion, the recovery (determined by comparing the concentration in the dialysis probe effluent with that of the medium, i.e., the percent recovery) depends primarily on the perfusion rate through the dialysis fiber. We perfused microdialysis probes (n = 5) at 0.5, 0.8, 1.0, 1.5, 2.0, and 2.5 μl/min with isotonic saline in a beaker that contained a bathing medium of isotonic saline (maintained at 37.5°C) and [3H]ANG II (49.2 Ci/mmol; DuPont-New England Nuclear; Boston, MA) at a concentration of 1 nCi/ml. At 1.0 μl/min, the rate that was used for the in vivo experiments, the percent recovery was 15.6 ± 2%. Our recovery rate of 15.6% was used in the final calculation of ISF values and thus represents an estimate of ISF levels, because diffusional exchange may differ between a beaker and a beating heart.

In vivo stability of our microdialysis probes was assessed by determining the concentration of the stable compound acetaminophen in the effluent from the probe in three rats (1 probe/rat) for five 1-h collections in each rat 5 days after insertion of the probe. Concentrations of acetaminophen were calculated using HPLC analysis of the dialysis probe inflow and effluent. The initial concentration in the infusate was considered to be 100%. We found that 85% of acetaminophen was detected in the effluent, which indicates that 15% diffused into the ISF, because the compound is not degraded under these conditions. This was a constant finding for the five 1-h perfusions for all three probes. Thus these...
experiments document that our dialysis probes did not become plugged or break down over the time course of the in vivo experiments.

Biochemical Assays of ISF

**ANG II concentrations.** Plasma ANG II concentrations were determined by a method we previously described that combines HPLC and RIA (1, 8, 10), and ISF ANG II samples were determined by direct RIA. Elution of standard ANG peptides under isocratic conditions by reverse-phase HPLC on a phenyl-silica gel column reveals clear resolution of ANG I, II, and III as well as ANG1–7 and ANG3–8 peptides as we have previously described (23). RIA of relevant peaks reveals detectable levels of ANG I and II in all ISF samples examined. Antibodies to ANG I and II are raised in our laboratory in New Zealand White rabbits immunized against peptides conjugated to poly-L-lysine as previously described (23). Cross-reactivity of anti-ANG I antiserum with ANG II and of anti-ANG II antiserum with ANG I was <0.5%. The sensitivity of the RIA for ANG I was 4 pg/ml; for ANG II, it was 2 pg/ml.

**BK concentrations.** ISF BK concentrations were determined using a standard RIA kit (Phoenix Pharmaceuticals; Mountain View, CA). For BK collections, ISF was collected immediately in an iced Eppendorf tube that contained 98% pure ethanol.

Biochemical Assays of Cardiac Tissue

**Heart chymase-like activity using ANG I as substrate.** LV myocardial samples were assayed for chymase-like activity as we have previously described (8, 10). Generated ANG II was quantitated using a reverse-phase Altima 5-μm phenyl-HPLC column. The peak area corresponding to a synthetic ANG II standard was integrated to calculate absolute ANG II formation. Chymase-like activity was defined as chymostatin-inhibitable ANG II formation and was expressed as nanomoles of ANG II formed per gram of tissue (wet weight) per minute.

**Cardiac ACE activity using hippuryl histidyl leucine as substrate.** Cardiac ACE activity was measured using an assay developed in our laboratory (8, 10, 22). According to this method, ACE is extracted from homogenized cardiac tissue with detergent, and the reaction product hippuric acid is isolated from the reaction mixture by reverse-phase HPLC, which thus eliminates interference from the detergent, the substrate hippuryl histidyl leucine, and unreacted reaction byproducts.

Quantitative evaluation of myocardial mast cells. The number of mast cells was quantitatively determined for the entire epicardial LV wall using Giemsa-stained paraffin sections. This method produces intense purple staining that is specific for mast cell granules. We examined 50–70 fields (each 122,330 μm²) using the ×20 objective of the microscope, and the number of mast cells per square millimeter was tabulated as previously described (8).

**Statistics**

All data are presented as means ± SE. A one-way ANOVA was used to compare morphometric, hemodynamic, ACE, and chymase activities; plasma ANG I and ANG II; and mast cell density between groups at the time of death and after 5 days of ACF. A one-way repeated-measures ANOVA was also used to compare changes in ISF ANG II and BK over time (baseline, 2 days, and 5 days) within groups. When significance was indicated, all pairwise multiple-comparison tests were performed using the Student-Newman-Keuls method. In cases where data failed to pass tests for normality of distribution and/or equal variance, a nonparametric ANOVA on ranks was performed using Dunn’s method to determine significant differences between treatments. All statistics were calculated using SigmaStat software (SPSS; Chicago, IL). A P value of <0.05 was required for significance.

**RESULTS**

**Morphometric and Hemodynamic Data**

After 5 days of ACF, there was a 45% increase in total heart weight-to-body weight ratio, which was significantly greater than in sham, sham + R, ACF + R, and ACF + R + HOE-140 animals (Table 1). The LV weight-to-body weight ratio was increased 30% in ACF vs. sham animals (P < 0.05), whereas the LV weight-to-body weight ratio did not differ among sham, ACF, ACF + R, and ACF + R + HOE-140 rats. RV weight-to-body weight ratio was increased in both ACF and ACF + R rats. RV weight-to-body weight ratio was decreased in both sham + R and ACF + R + HOE-140 compared with ACF rats. As expected, there was a significant decrease in mean arterial pressure due to the ACF that did not differ among ACF, ACF + R, and ACF + R + HOE-140 rats (Table 2). In both treated and untreated rats, LV end-diastolic pressure did not differ from sham rats. Peak +LV dP/dt did not differ in sham vs. ACF and ACF + R rats; however, peak +LV dP/dt increased significantly in ACF + R + HOE-140 compared with ACF rats. As expected, there was a significant increase in mean arterial pressure due to the ACF that did not differ among ACF, ACF + R, and ACF + R + HOE-140 rats (Table 2). In both treated and untreated rats, LV end-diastolic pressure did not differ from sham rats. Peak +LV dP/dt did not differ in sham vs. ACF and ACF + R rats; however, peak +LV dP/dt increased significantly in ACF + R + HOE-140 compared with ACF and sham + R rats.

ACF + R + HOE-140 treatment resulted in a significantly different remodeling pattern compared with ACF + R that was manifested by higher wall thickness-to-chamber diameter ratios and smaller chamber diameters (Fig. 1). In addition, wall thickness was increased in ACF + R + HOE-140 rats compared with both sham and ACF + R rats, whereas wall thickness...
did not differ in sham vs. ACF rats. These differential changes in LV remodeling are demonstrated by the LV cross sections provided in Fig. 2.

**Plasma and ISF ANG II and ISF BK Levels**

In control rats, ISF ANG II and BK levels were measured each day over 5 days and did not differ over this period of time (Fig. 3). In sham rats, ACE-inhibitor treatment resulted in a significant increase in ISF BK at 5 days, whereas ANG II levels did not change over time. In ACF rats, there was a significant increase in ISF ANG II and ISF BK at 5 days vs. baseline (Figs. 4 and 5, respectively). In ACF/H11001 rats, there was no increase in ANG II at 5 days, whereas ISF BK levels were significantly increased at both 2 and 5 days compared with baseline. In ACF/H11001/H11001 HOE-140 rats, ISF ANG II levels did not differ from baseline at 5 days. However, there was a fourfold increase in ISF BK that was greater than both baseline and day 2 ISF BK levels.

In ACF rats, plasma ANG II levels increased 80% vs. sham rats (41 ± 2 to 75 ± 6 pg/ml; *P < 0.05), whereas ANG I levels were unchanged (222 ± 12 vs. 166 ± 11 pg/ml; Fig. 6). In ACE rats, treatment with ACE inhibitor significantly decreased plasma ANG II levels to sham levels (44 ± 5 pg/ml; *P < 0.05), and ANG II remained at sham levels with the addition of HOE-140 to ACE inhibitor. Plasma ANG I levels were increased to 702 ± 84 pg/ml compared with sham, ACF, and ACF + R rats (*P < 0.05).

**LV ACE and Chymase Activities and Mast Cell Numbers**

There was a tendency toward an increase in LV ACE and chymase activities at 5 days after ACF induction and a reduction with ramipril treatment, which reflects the changes in ISF ANG II. However, ACE and chymase activities differed only in ACF vs. sham rats (Fig. 7). There was a significant increase in mast cell density in ACF and ACF/H11001 rats at both 2 and 5 days after ACF. In ACF/H11001/H11001 HOE-140 rats, mast cell density decreased to levels that were even below sham rats (Fig. 8).

**DISCUSSION**

In the current investigation, acute volume overload resulted in a significant increase in ISF ANG II and BK. Treatment with ACE inhibitor further augmented ISF BK and decreased ANG II levels resulting in an attenuation of the LV hypertrophic response. Addition of the BK2 receptor antagonist to ACE inhibitor resulted in a LV mass that was similar to ACE inhibitor alone in response to volume overload. However, blockade of the BK2 receptor produced a more concentric LV hypertrophy as evidenced by a thicker wall and smaller chamber diameter. Further, there was a significant increase in mast cell density in ACF and ACF + R rats that was decreased below sham in ACF + R + HOE-

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**Table 2. Hemodynamic data**

<table>
<thead>
<tr>
<th></th>
<th>Heart Rate, beats/min</th>
<th>Mean Arterial Pressure, mmHg</th>
<th>LV End-Diastolic Pressure, mmHg</th>
<th>LV + dP/dt, mmHg/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>327 ± 11</td>
<td>113 ± 6</td>
<td>6.1 ± 0.9</td>
<td>2,892 ± 179</td>
</tr>
<tr>
<td>ACF</td>
<td>343 ± 10</td>
<td>86 ± 3*</td>
<td>9.7 ± 1.9</td>
<td>2,304 ± 118</td>
</tr>
<tr>
<td>Sham + R</td>
<td>310 ± 12</td>
<td>86 ± 4*</td>
<td>8.1 ± 1.0</td>
<td>2,218 ± 111</td>
</tr>
<tr>
<td>ACF + R</td>
<td>336 ± 12</td>
<td>76 ± 4*</td>
<td>7.6 ± 1.4</td>
<td>3,795 ± 547</td>
</tr>
<tr>
<td>ACF + R + HOE-140</td>
<td>375 ± 26</td>
<td>81 ± 7*</td>
<td>9.6 ± 2.6</td>
<td>6,551 ± 523†‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 vs. sham; †P < 0.05 vs. ACF; ‡P < 0.05 vs. sham R.
140 rats. These results suggest a potentially important interaction of mast cells and BK in modulating the effects of ACE inhibition on the pattern of LV remodeling in the acute phase of volume overload in rats.

To our knowledge, the increase in ISF BK with acute hemodynamic stress of volume overload is a novel finding. It is well appreciated that acute ischemia results in an increase in BK across the coronary vascular bed and within the cardiac interstitium of the heart (18, 27). However, cardiac kallikrein and kininogen are released from isolated rat hearts in response to acute volume overload (26), which suggests an increase in cardiac kinin-generating activity with acute hemodynamic stress. These results are consistent with a local kallikrein-kinin system in the heart that is capable of synthesizing and releasing kinins (25). The results of the current investigation cannot rule out cardiac uptake of BK due to the substantial kinin-forming activity in plasma. Nevertheless, ISF BK increased with the addition of ACE inhibitor and markedly with the addition of HOE-140; the latter is suggestive of a myocardial tissue effect on the BK2 receptor.

ACE inhibition prevented the increase in the LV weight-to-body weight ratio, which is consistent with the expected effects attendant with a decrease in ISF ANG II and an increase in ISF BK. Previous studies of LV pressure overload (19) and myocardial infarction (20, 41) in rats demonstrate that BK2 receptor antagonism in part reverses the hemodynamic and antihypertrophic effects of ACE inhibitor. However, in the present investigation, addition of the BK2 receptor antagonist did not increase blood pressure nor did it reverse the antihypertrophic effect of the ACE inhibi-
Fig. 4. ISF ANG II at baseline (base) and 2 and 5 days after sham + R (A), ACF (B), ACF + R (C), and ACF + R + HOE-140 (D) treatments. *P < 0.05 vs. baseline.

Fig. 5. ISF BK at baseline and 2 and 5 days later: sham + R (A), ACF (B), ACF + R (C), and ACF + R + HOE-140 (D) treatments. *P < 0.05 vs. baseline; †P < 0.05 vs. day 2.
The addition of HOE-140 resulted in an increase in peak LV \( \frac{dP}{dt} \) compared with ACF alone. This effect may in part be attributed to the greater wall thickness-to-chamber diameter ratio that in the face of similar systemic arterial pressure resulted in a lower wall stress and marked increase in peak LV \( \frac{dP}{dt} \).

Although ACE activity trended upward, it did not increase to a significant extent after 5 days of ACF. This is consistent with previous studies where LV ACE correlated positively with the severity of heart failure in dogs (8, 10) and in human patients (43). Nevertheless, ACE inhibitor treatment normalized a twofold increase in ISF ANG II during ACF, which suggests that ACE is the predominant ANG II-forming mechanism in rat hearts. However, ISF ANG II levels did not decrease below normal, and thus this study cannot address the contribution of chymase to ANG II formation in the heart during the acute stages of volume overload.

The addition of HOE-140 did not affect the attenuation of the ISF ANG II levels by ramipril but did result in a more concentric pattern of LV remodeling without affecting the amount of cardiac hypertrophy. This result suggests that activation of the BK2 receptor is important in the eccentric remodeling in response to volume overload independent of ISF ANG II levels. In support of this hypothesis, we studied LV remodeling in response to ACF in heterozygous \([+/−]\) ACE knockout mice that possessed only one functional ACE gene and 50% of normal ACE activity compared with wild-type mice (30). There was a decrease in LV wall thickness-to-LV diameter ratio in \([+/−]\) mice compared with wild types (30) in the presence of similar LV ANG II levels after 1 mo of ACF. In subsequent studies in

![Fig. 6. Plasma ANG II (A) and ANG I (B) concentrations in rats 5 days after sham surgery, ACF, ACF + R, and ACF + R + HOE-140. *\( P < 0.05 \) vs. sham; †\( P < 0.05 \) vs. ACF; ‡\( P < 0.05 \) vs. ACF + R.](image)

![Fig. 7. LV ANG-converting enzyme (ACE) and chymase activities in sham (n = 6), ACF (n = 10), sham + R (n = 6), ACF + R (n = 6), and ACF + R + HOE-140 (n = 8) rats. LV ACE activity did not differ from sham and at 48 h and 5 days in ACF rats. In contrast, chymase activity increased 50% at 5 days in ACF rats. *\( P < 0.05 \), 5-day ACF vs. sham + R.](image)

![Fig. 8. LV myocardial mast cell numbers after 2 days of ACF (n = 6) and 5 days each of ACF (n = 6), ACF + R (n = 6), and ACF + R + HOE-140 (n = 6) vs. sham (n = 6) rats. *\( P < 0.05 \) vs. sham; †\( P < 0.05 \) vs. 5-day ACF; ‡\( P < 0.05 \) vs. 2-day ACF; ††\( P < 0.05 \) vs. ACF + R.](image)
mice with an extra ACE copy and higher ACE activity, we found greater wall thickness-to-diameter ratio compared with wild types despite similar blood pressure and LV ANG II levels across genotypes (unpublished observations). Taken together, the differential expression of ACE and its subsequent effect on tissue BK and BK2 receptors could mediate a more concentric or eccentric LV remodeling pattern in volume overload.

Another important component in this early phase of volume overload is an acute inflammatory response manifested by an increase in mast cell density. Mast cells provide a heterogeneous source of cytokines, chemokines, proinflammatory mediators, and, in particular, chymase, which itself can activate matrix metalloproteinases (MMPs) (14, 32). A recent study by Chancey et al. (6) demonstrated that chemically induced mast cell degranulation in a normal rat heart produces an almost-immediate substantial activation of MMP-2, marked degradation of interstitial fibrillar collagen (i.e., ~50% reduction in collagen volume fraction), and ventricular dilation. In the current study, there was a significant increase in mast cell density in ACF rats that was not attenuated by ACE inhibitor treatment. This could augment the proinflammatory effects of BK and lead to the observed chamber dilatation. It is now well appreciated that ACE inhibitor potentiates the action of the BK2 receptor (24). BK2 receptor antagonist combined with ACE inhibitor reversed the eccentric remodeling in addition to decreasing the mast cell density below sham levels.

In addition to its well-appreciated vasodilatory effects, BK also has important proinflammatory effects including leukocyte accumulation (4). It is of interest that in mice devoid of the BK2 receptor, there is a decrease in macrophage accumulation in the renal interstitium in response to ureteral obstruction (33). BK has also been shown to stimulate lung fibroblasts to release chemotactic activity for both neutrophils and monocytes (17). BK acting on its BK2 receptor has been shown to play a critical role in mediating mast cell dependent damage in a rat model of pleural inflammation (2). In our rats, ISF BK was increased at 2 days with ACE inhibitor treatment. This could augment the proinflammatory effects of BK and lead to the observed chamber dilatation. It is now well appreciated that ACE inhibitor potentiates the action of the BK2 receptor (24). BK2 receptor antagonist combined with ACE inhibitor reversed the eccentric remodeling in addition to decreasing the mast cell density below sham levels. These combined effects would appear to have improved the short-term adaptive response to the volume overload as evidenced by a greater wall thickness-to-diameter ratio and a higher peak LV +dP/dt.

These results suggest that alterations in the balance of ANG II and inflammatory mediators such as BK and mast cells may play an important role in ventricular remodeling in response to acute volume overload. In the present investigation, ACE inhibitor was started immediately after the hemodynamic stress. In all other studies where HOE-140 reversed the beneficial effects of ACE inhibition, drugs were started 1 wk (20) or 2 mo (41) after myocardial infarction in rats. Treatment at this point in time has shown that the kinin-augmenting effect of ACE inhibition contributes to the beneficial reduction in myocardial collagen at these later stages in this model. However, preliminary data in our (36) and in other (3) labs reveal that extracellular matrix degradation occurs as early as 6 h after induction of volume-overload stress. Thus it is possible that early ACE inhibitor therapy could potentiate matrix degradation and result in greater chamber dilation at 5 days. Future studies will relate ISF ANG II and BK levels at various stages of volume overload to mast cell density, MMP expression and activity, extracellular matrix homeostasis, and LV remodeling and function.

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DISCLOSURES

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