Distribution and Functional Significance of Cardiac Angiotensin Converting Enzyme in Hypertrophied Rat Hearts

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Background. The intracardiac conversion rate of angiotensin (Ang) I to Ang II and the expression of angiotensin converting enzyme (ACE) mRNA are amplified in rat hearts with left ventricular hypertrophy (LVH). To examine whether the accelerated intracardiac Ang II generation in LVH is related to an induction of cardiac ACE, we studied localization and function of cardiac ACE in hypertrophied rat hearts using specific ACE inhibitors.

Methods and Results. Cardiac ACE was localized and quantified in hearts from male Wistar rats with LVH due to chronic experimental aortic stenosis and from control rats. With the ACE inhibitor 125I-351A, a derivative of lisinopril, as a radioligand on coronal sections of LVH and control hearts, in vitro autoradiography demonstrated ACE binding in aorta, coronary arteries, atria, and ventricles of both groups. Quantitative analyses revealed that ACE density (counts per minute per cross-sectional area of tissue) was twofold higher within the myocardium of hypertrophied left ventricles compared with controls (p<0.005). Quantitative morphometry demonstrated a modest increase in the fractional volume of myocytes as well as capillary volume without an increase in the fractional volume of endothelial cells in left ventricular tissue from aortic stenosis rats. These data suggest that an increase in endothelial cell volume per se cannot alone account for the observed doubling of ACE density and support an upregulation of ACE production in hypertrophied tissue. The role of cardiac ACE in intracardiac conversion of Ang I to Ang II and its specific inhibition was studied in isolated, isovolumic beating, buffer-perfused LVH and control hearts. Biochemical conversion rates as well as functional changes in response to 3×10^{-7} M Ang I were examined in the absence or presence of the ACE inhibitor enalaprilat (4×10^{-6} M). After a brief stabilization period, groups of LVH and control hearts were subjected to the following infusion protocols: 15 minutes of vehicle followed by 30 minutes of Ang I plus vehicle, 15 minutes of enalaprilat followed by 30 minutes of Ang I plus enalaprilat (enal/Ang I), or 45 minutes of vehicle only to allow comparison with a time control. Intracardiac Ang I→Ang II conversion rate was fourfold higher in LVH than in control hearts (p<0.05). Infusion of enalaprilat reduced the intracardiac Ang I→Ang II conversion rate in LVH hearts by 70% (p<0.05 versus Ang I). At similar levels of constant coronary flow per gram, Ang I increased coronary perfusion pressure by 23±5 mm Hg (p<0.01 versus vehicle) in LVH hearts and by 36±10 mm Hg (p<0.005 versus vehicle) in control hearts. When enalaprilat was infused with Ang I, the increase in perfusion pressure was limited to 5±5 mm Hg (NS versus vehicle) in LVH hearts and 12±3 mm Hg (p<0.05 versus vehicle) in control hearts and was significantly lower than in hearts infused with Ang I only (p<0.05 in LVH and p<0.05 in control hearts, respectively). Systolic function was not affected by either infusion protocol. In contrast, Ang I infusion was associated with diastolic dysfunction. In LVH hearts, left ventricular end-diastolic pressure (LVEDP) increased from 10±1 mm Hg at baseline to 25±2 mm Hg at the end of the Ang I infusion (p<0.001 versus vehicle), which was inhibited by infusion of enalaprilat. In control hearts, there was a lesser increase in LVEDP from 10±1 mm Hg to 15±1 mm Hg in response to Ang I (p<0.05 versus LVH). Control hearts treated with enalaprilat with Ang I displayed no increase in LVEDP.

Conclusions. These observations indicate that ACE protein is increased within the myocardium of LVH hearts, extending recent findings of increased cardiac ACE activity and mRNA levels in this model of pressure-overload LVH in the rat. Blockade of the enzyme by an ACE inhibitor decreases intracardiac Ang I→Ang II conversion rate and prevents the functional changes of Ang I→Ang II activation otherwise seen in LVH hearts. Taken together, these data suggest that ACE is a key enzyme involved in the enhanced conversion of Ang I to Ang II in the intact hypertrophied rat heart. (Circulation 1993;87:1328–1339)

KEY WORDS • angiotensin I • angiotensin II • diastole • angiotensin converting enzyme • endothelium • enalaprilat
Current data support the existence of an angiotensin (Ang) II–forming pathway in the heart. The presence of angiotensinogen, renin, angiotensin converting enzyme (ACE), and Ang II receptors in cardiac tissue has been demonstrated, at both mRNA and protein levels, suggesting local synthesis of these components of the renin-angiotensin system in the heart. Although the physiological roles of this local renin-angiotensin system are not yet fully elucidated, it is conceivable that locally synthesized Ang II might contribute to cardiac effects of the octapeptide, such as coronary vasoconstriction, positive inotropy, and negative lusitropy, as well as induction of myocyte growth.

The final step of the enzymatic cascade leading to Ang II generation in the heart is facilitated by ACE.2,7,9 Tissue ACE is localized in the epicardial coronary vascularature and valve leaflets and throughout the myocardium of atria and ventricles.10,11 The activity of cardiac ACE may be increased in various forms of myocardial disease. Cardiac ACE is elevated in myocardial tissue from rat hearts with compensated experimental heart failure after left ventricular myocardial infarction.12,13 Furthermore, we have shown that cardiac ACE mRNA levels and activity are induced in chronic pressure-overload left ventricular hypertrophy (LVH) due to experimental aortic stenosis in the rat. Increased cardiac ACE activity has been associated with increased local generation of Ang II when isolated hypertrophied rat hearts were perfused with Ang I.7 Furthermore, the local Ang II generation was followed by coronary vasoconstriction and exaggerated diastolic dysfunction in hearts with LVH.7 The precise tissue site of ACE induction in LVH hearts has not yet been localized.

Pharmacological blockade of cardiac ACE by ACE inhibitors, such as captopril, ramiprilat, SQ-20881, and cilazaprilat, can decrease the intracardiac Ang I–to–Ang II conversion rate and the cardiac tissue content of Ang II in normal rabbit or rat hearts.4,14–16 Accordingly, it has been hypothesized that cardiac ACE inhibition contributes to the overall pharmacodynamic profile of ACE inhibitors in myocardial disease as well.1,2,15 However, other non-ACE peptidases might interfere with the activity of the cardiac renin-angiotensin system. A recently discovered non-ACE chymase peptidase, found in the cardiac membrane preparations from human hearts, has been shown to generate Ang II from Ang I in an in vitro system.17,18 The in vivo activity and physiological significance of the non-ACE chymase peptidase is not yet elucidated.

The present investigation was performed to differentiate the relative contributions of ACE and non-ACE pathways on the amplified cardiac Ang I conversion in hypertrophied rat hearts and the consequence of these pathways on left ventricular function. The biochemical Ang I–conversion rate and the functional effects of Ang I infusion were studied in the absence and presence of enalaprilat. Furthermore, ACE was localized and quantified in hypertrophied left ventricular myocardium employing radio-labeled 351A, a derivative of lisinopril, and quantitative morphometry of left ventricular tissue was performed to determine if a simple increase in the fractional volume of microvascular endothelial cell compartment could account for the increase in left ventricular ACE activity in the hypertrophied hearts.

Methods

Preparation of Animals

Male Wistar rats were obtained from the Charles River Breeding Laboratories. Aortic stenosis was created in weanling rats (body wt, 100 g; age, 3–4 weeks) by placing a stainless-steel clip of 0.6-mm internal diameter on the ascending aorta via a thoracic incision. Age-matched control animals underwent a left thoracotomy. The rats subsequently were fed standard rat chow (Purina) and water ad libitum. Animals were used for experimentation 9 weeks after surgery because morphological studies of left ventricular tissue from aortic stenosis versus sham-operated control hearts revealed significant myocyte hypertrophy (cell diameter, 17.8±0.6 versus 11.8±0.9 µm, p<0.01) but absent or minimal proliferation of connective tissue at this stage of pressure overload.

Quantitation of Myocardial ACE by In Vitro Autoradiography

The methods for in vitro autoradiography of ACE have been previously described.10 Tissue for autoradiographic study was obtained immediately after killing the rats. The heart was dissected free, snap-frozen, and maintained at −20°C until processed. Coronal sections (20 µm) were cut through the left ventricle, maintained in a cryostat at −20°C, thaw-mounted onto gelatin-coated slides, and dried in a desiccator for 2 hours at 4°C. Sections were incubated in 10 mM sodium phosphate buffer (pH 7.4) containing 150 mM sodium chloride, 2 g/L bovine serum albumin, and 0.3 µCi/mL 125I-351A for 1 hour at 20°C. Nonspecific binding was determined in parallel incubations with buffer containing 1 mM EDTA and 1 µM lisinopril. After incubation, the sections were transferred through four successive 1-minute rinses in buffer at 0°C, dried under cold air, and exposed to AGFA-Scopix CR3B x-ray film in cassettes for 3 days. X-ray films were processed in a Kodak RPXomat automatic developer, and the optical density of autoradiographs was quantitated using an EYE-COM model 850 image analysis system (Spatial Data Systems, Springfield, Va.) coupled to a Dec 11-23 LSI computer. Optical density was calibrated by fitting curves with a computer using standards carried through the above procedure.

Three rats with aortic stenosis and three sham-operated control rats were studied. Serial sections were taken from the left ventricle, with 12 sections analyzed.
from each heart. Autoradiograph density (density of signal reflects the relative density of binding sites for the radiolabeled ACE inhibitor per cross-sectional area) of the left ventricle was measured in all sections under standard conditions to derive an optical density index (DPM/mm²) for each region of interest. Data from each section were pooled, and the mean was calculated for the 12 sections from each rat. The region of interest selected in each section specifically excluded the valvular structures.

Quantitative Tissue Morphometry of Capillary Volume Fraction

Tissue morphometry was performed using a modification of the methods of Anversa et al.19,20 In brief, hearts from three rats with aortic stenoses and three sham-operated control rats were studied. Hearts were perfused on the perfusion apparatus via a cannula in the aortic stumps at a perfusion pressure of 80 mm Hg for 3 minutes with buffer and then arrested in diastole with KCl. The coronary vasculature was then perfused at the same constant pressure with a solution containing 2% paraformaldehyde and 2.5% glutaraldehyde for 15 minutes for fixation. Three tissue blocks from the lateral mid free wall of each ventricle of each of three animals of LVH and sham groups were embedded in PolyBed 812 (Polysciences, Warrington, Pa.) and trimmed for thin sectioning to obtain areas of tissue with transversely sectioned myofibers. Five electron photomicrographs of random areas of ultrastrains of each tissue block were printed at x5,000, calibrated with a deflection grating replica magnification standard (EF Fulham, Inc., Schenectady, N.Y.), and analyzed morphometrically with a superimposed rectangular grid consisting of 221 sampling points. Quantitative determination of the fractional volumes (Vv) of myocardium composed of myocytes, capillary lumen, capillary endothelial cells, interstitial cells, and extracellular matrix was made by counting the fraction of sampling points (Pv) overlaying each of these components (Vv = Pf). The numbers of capillary profiles in the photographs were counted to estimate their numerical densities. Results are presented as mean ± SD values computed from the average measurements obtained from each rat.

Perfusion Technique

The isolated isovolumic working rat heart preparation is described in detail.21 Rats were injected intraperitoneally with 1.0–1.5 mL sodium pentobarbital (15 mg/mL), and the thorax was opened rapidly. Within 20 seconds, the hearts were placed into a water-jacketed constant temperature chamber (37°C), and the coronary arteries were perfused by a constant flow pump (Masterflex; Cole-Parmer, Inc.) through a short cannula inserted into the aortic root just below the level of the aortic clip. The perfusate consisted of modified Krebs-Henseleit buffer of the following composition (in mM): NaCl 118, KCl 4.7, CaCl2 2.0, KH2PO4 1.2, MgSO4 1.2, NaHCO3 25, lactate 1.0, and glucose 5.5. The perfusate was equilibrated with a 5% CO2–95% O2 gas mixture that achieved a PO2 of approximately 550 mm Hg and pH 7.35–7.40.

A small cannula was inserted into the left ventricular apex to vent any Thebesian drainage. The pulmonary artery was drained to completely collect coronary venous effluent and to empty the right ventricle. A thermistor and a pacing electrode were inserted into the right ventricle via the right atrium, and the venae cavae were ligated. A collapsed latex balloon, slightly larger than the left ventricular chamber such that no measurable pressure was generated over the range of volumes used, was placed into the left ventricle, and left ventricular pressure was measured via a Statham P23Db pressure transducer (Statham Instruments, Inc., P.R.) connected to the balloon via a short length of stiff polyethylene tubing. The damping characteristics and the natural resonant frequency response of this system22 satisfy the range shown by Falsetti et al.23 to be required for accurate measurement of left ventricular pressure and its first derivative. To assess left ventricular chamber distensibility, left ventricular balloon volume was initially adjusted to 10 mm Hg in both groups, and this balloon volume was maintained constant so that an increase in left ventricular end-diastolic pressure (LVEDP) signified a decrease in diastolic chamber distensibility.21,22 Coronary perfusion pressure was measured from a side-arm of the aortic perfusion cannula connected to a Statham P23Db pressure transducer. The coronary flow rate was measured with timed samples of coronary venous effluent collected from the pulmonary artery cannula. In hearts from the rats with chronic aortic stenosis (LVH groups), coronary flow was adjusted to achieve a coronary perfusion pressure of 100 mm Hg and then was fixed at that level of flow throughout the subsequent experiment. In the control groups, coronary flow was adjusted to achieve a coronary perfusion pressure of 80 mm Hg and then was fixed at that level throughout the experiment. These differing levels of coronary flow and initial coronary perfusion pressures were selected in recognition of the difference between the in vivo mean coronary perfusion pressures to which the control and LVH groups were chronically exposed and because prior studies showed that this approach would achieve comparable myocardial perfusion flow rates per gram of left ventricular weight and an aerobic pattern of myocardial lactate extraction24 in both groups. The hearts were paced at 4 Hz throughout the experiment.

Experimental Protocol

The LVH and control hearts were distributed randomly to three infusion protocols, respectively. In the first protocol, LVH (n = 7) and control (n = 8) hearts received vehicle infusion for 15 minutes followed by infusion of 3×10−7 M Ang I (Sigma Chemical Co., St. Louis, Mo.) for 30 minutes (Ang I). In the second protocol, LVH (n = 7) and control (n = 8) hearts received enalaprilat infusion (4×10−8 M) for 15 minutes before and together with 30 minutes of 3×10−7 M Ang I infusion (enal/Ang I). In the third protocol, LVH (n = 5) and control (n = 5) hearts were infused with vehicle for 45 minutes to establish a time control (vehicle). At the end of each infusion period, measurements of left ventricular pressure, coronary perfusion pressure, and coronary flow were made. During the final 5 minutes of the Ang I infusion period, the coronary venous effluent was collected in 4% trifluoroacetic acid. The samples were immediately frozen and stored at −20°C for subsequent processing.
To study whether the increase in LVEDP in Ang I-infused LVH hearts was indirectly mediated by the increase in coronary perfusion pressure, the effects of incremental levels of coronary flow and coronary perfusion pressure on LVEDP were examined in five additional LVH hearts. Hearts were perfused under identical conditions as mentioned above, except that coronary flow was increased in 5-minute intervals to simulate the magnitude and time course of the levels of coronary perfusion pressure reached with Ang I infusion.

Biochemical Analysis of Ang I-to-Ang II Conversion in the Perfused Hearts

The coronary venous effluent samples were heated to boiling, and precipitated proteins were removed by centrifugation. The supernatant was partially purified with RP 18 SEP-PAK cartridges (Waters Associates, Milford, Mass.) as previously described.7 After washing with 0.01 M trifluoroacetic acid, the SEP-PAK cartridges were eluted with 80% methanol in 0.01 M trifluoroacetic acid. The eluate was lyophilized and then redissovled in 0.02 M acetic acid and subjected to high-performance liquid chromatography (HPLC). Reverse-phase HPLC was done using a Varian 5000 solvent delivery system combined with a Spectroflow 757 variable ultraviolet monitor (LKB Instruments, Parmaus, N.Y.) tuned to 216 nm. The data processing was assisted using an Apple 2E personal computer with CHROMATChART software (Interactive Microwave, Inc., State College, Pa.). The angiotensins were separated on an ultrtropac column (Sperisorb ODS—2.3 μm; 4.6×50 mm; LKB Instruments). The solvent consisted of 40% methanol in 10 mM sodium acetate, pH 5.6 (solvent A), and 80% methanol in 10 mM sodium acetate, pH 5.6 (solvent B). The gradient was B=0% at time 0 and B=100% at 30 minutes. The flow rate was 1 mL/min. The fractions were collected in polypropylene test tubes with a collection time per fraction of 30 seconds. Synthetic angiotensins were used for calibration of the HPLC column. The recovery was 83% for Ang I and 94% for Ang II. The fractions corresponding to the retention times of synthetic Ang I, II, and III were pooled. After lyophilization, samples were redissolved in 0.2 mL of 0.02 M acetic acid diluted with radioimmunoassay (RIA) buffer (10 mM Tris, pH 7.4, with 1 mg/mL bovine serum albumin), and RIA performed as previously described.7 The sensitivity of this assay is 0.1–1.2 ng/tube. Because RIA was always performed after HPLC, the cross-reactivity of the Ang II antibody with Ang I or nonangiotensin peptides was avoided. The fractional conversion rate was calculated as [(Ang II, M)/(Ang I+Ang II, M)]×100.

Statistical Analysis

All values are expressed as the mean±SEM. The statistical analysis of differences observed between the LVH and control groups in regard to myocardial ACE density and fractional volumes of myocardial cellular components was done using Student's t test for unpaired data. The statistical analysis of differences observed between LVH Ang I, LVH enal/Ang I, and Ang I groups in regard to fractional conversion of Ang I to Ang II was done using ANOVA comparison and Fisher's exact test for post hoc analyses. Statistical signiﬁcance was accepted at the level of p<0.05. Statistical analysis of the effects of Ang I infusion, Ang I plus enalaprilat infusion, or vehicle infusion on the LVH and control group was done using ANOVA for repeated measures.

Results

Extent of Hypertrophy

The LVH group had moderate LVH relative to the control group, with the mean left ventricular wet weight increased 38% above control (1.47±0.05 g, p<0.001). The average body weight was slightly lower in the LVH groups (334±13 versus 386±8 g, p<0.05), and the mean left ventricle-to-body weight ratio was increased 64% above the control group (4.5±0.2 versus 2.7±0.1 g/kg, p<0.001). There were no significant differences in left ventricular weights between the subgroups treated with the various infusion protocols.

Quantitative Analysis of Myocardial ACE Density in LVH Hearts

ACE was localized by in vitro autoradiography of coronal sections of LVH and control hearts. Binding of the radiolabeled ACE inhibitor was detected throughout the myocardium of left and right ventricles (Figure 1) as well as in aorta and coronary vessels. Quantitative analysis of coronal sections of three LVH and three control rat hearts revealed a twofold increase in left ventricular myocardial ACE density in LVH compared with control hearts (p<0.005) (Figures 1 and 2).

Quantitative Morphometry: Growth of Microvasculature

Quantitative determination of the fractional volumes (Vv) of left ventricular myocardium composed of myocytes, capillary lumen, capillary endothelial cells, interstitial cells, and extracellular matrix was performed (Table 1). Representative electron photomicrographs are shown in Figure 3. In left ventricular tissue from the aortic stenosis rats relative to controls, there was a modest increase in the fractional volume of myocytes (5.3%) as well as in the fractional capillary lumen volume (26.3%) without an increase in the fractional volume of endothelial cells or interstitial cells.

Fractional Conversion of Ang I to Ang II in the Isolated, Perfused Heart

Neither of the angiotensins was found in the coronary venous effluent under baseline conditions when hearts were perfused with buffer solution alone. In response to perfusion with 3×10−7 M Ang I, the intracardiac conversion rate of Ang I to Ang II in the perfused hearts was 12.1±6.3% in the LVH group and 2.6±1.3% in the control group (p<0.05). The fractional conversion of Ang I was assessed in LVH hearts after the addition of enalaprilat (4×10−8 M) 15 minutes before and together with Ang I infusion. In the presence of enalaprilat, the conversion rate decreased to 3.0±1.2% in the LVH hearts (p<0.05).

Coronary and Left Ventricular Hemodynamics in Response to Ang I

By study design, coronary flow was adjusted at baseline to achieve a similar coronary flow per gram left
ventricular weight in LVH and control groups (Table 2). Flow rate then was held constant during the remainder of the experiment such that changes in coronary perfusion pressure are indicative of changes in coronary vascular resistance. Forty-five minutes of vehicle infusion or 15 minutes of enalaprilat infusion in the absence of exogenous Ang I had no significant effect on coronary perfusion pressure in either group. In response to infusion of Ang I, there was an increase in coronary vascular resistance in both LVH and control hearts (Figure 3). When enalaprilat was infused together with Ang I, the increase in coronary vascular resistance was significantly diminished. In LVH hearts perfused with enal/Ang I, coronary perfusion pressure was not significantly different from hearts perfused with vehicle only (Figure 4).

At baseline, left ventricular peak systolic pressure was higher in the LVH hearts, whereas developed pressure per gram left ventricular tissue was higher in control hearts (Table 1). Both LVH and control hearts displayed a minimal impairment of systolic function during the 45 minutes of vehicle infusion. Developed pressure per gram of left ventricular tissue in Ang I– or Ang I plus enalaprilat–infused hearts was not significantly different from the respective vehicle time-control group in either LVH or control groups (Figure 5).

By study design, isovolumic LVEDP was adjusted to 10 mm Hg at baseline in both LVH and control groups. Forty-five minutes of vehicle infusion or 15 minutes of enalaprilat infusion in the absence of Ang I infusion had no significant effect on LVEDP in either group. In control hearts, Ang I infusion was associated with a
slight increase of LVEDP to 15±1 mm Hg (p<0.05, Figure 6). In contrast, when enalaprilat was infused with Ang I, no significant difference in LVEDP was noted between enal/Ang I and vehicle-infused control groups. In LVH hearts, Ang I infusion was associated with a marked increase of LVEDP to 25±2 mm Hg (p<0.005 versus vehicle, and p<0.05 versus control/Ang I) (Figure 6). Parallel infusion of enalaprilat significantly prevented the exaggerated increase of LVEDP in LVH hearts infused with Ang I (p<0.01 versus LVH/Ang I) such that LVEDP was not different from vehicle-infused LVH hearts (Figure 6).

**Effect of Elevated Coronary Perfusion Pressure on LVEDP**

In an additional protocol, we studied the question as to whether the severe increase of LVEDP in LVH hearts infused with Ang I was mediated by the parallel increase of coronary perfusion pressure. Five LVH hearts were perfused at increasing levels of coronary flow to gradually increase coronary perfusion pressure over the same time course and to similar and higher levels as that observed in response to Ang II. An increase of coronary perfusion pressure comparable to the maximal increase seen in LVH hearts infused with Ang I (Δ30 mm Hg) was associated with a small but significant increase of LVEDP by about 3–4 mm Hg (Figure 7). However, this increase in LVEDP was significantly lower than the increase in LVEDP observed in Ang I–infused LVH hearts (Δ15 mm Hg, p<0.01).

**Discussion**

Increasing evidence suggests that the renin-angiotensin system is involved in the pathophysiology of cardiac pressure-overload hypertrophy. Ang II has been shown to stimulate growth of cardiac myocytes.25–27 Treatment with various ACE inhibitors, on the other hand, can prevent or diminish myocardial hypertrophy in experimental models of cardiac pressure overload, volume overload, or myocardial infarction.28–32 Furthermore, treatment with ACE inhibitors may regress cardiac hypertrophy in patients with chronic pressure overload33 in association with a favorable effect on diastolic function.34 Interestingly, even in the absence of an activated circulating renin-angiotensin system,3,12,35,36 the pharmacological effects of ACE inhibitors are sustained, suggesting a contribution of the tissue renin-angiotensin system. In support of this hypothesis, measurements of cardiac angiotensinogen, ACE mRNA levels, or tissue Ang II concentrations indicate that the activity of the tissue renin-angiotensin system is enhanced in experimental cardiac hypertrophy.3,7,12,37-39 Studies on Ang II receptors, on the other hand, indicate a decrease in Ang II–binding sites in this aortic-banded rat model of pressure-overload hypertrophy, which might reflect a down-regulation of the Ang II receptor by chronically elevated tissue Ang II concentrations (Reference 7 and S.S. Tang, unpublished observations). There is evidence that inhibition of cardiac ACE is sufficient to prevent hypertrophy in the aortic banded rat model used in the present study,40 but this effect is not observed in all models of cardiac hypertrophy.28–30,41,42 In this study, we used ACE inhibitors as a tool to study the distribution of cardiac ACE as well as the role of the enzyme in intracardiac conversion of Ang I to Ang II in hypertrophied rat hearts.

The major findings of our studies are that the fractional conversion of Ang I to Ang II is amplified in isolated beating hearts with LVH; ACE inhibition reduces the intracardiac Ang I conversion and functional related changes in coronary resistance and diastolic function of isolated, perfused LVH hearts; and ACE

**Table 1. Left Ventricular Quantitative Morphometry: Volume Fraction of Myocardial Components**

<table>
<thead>
<tr>
<th>Component</th>
<th>LVH</th>
<th>Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocytes (%)</td>
<td>76.8±1.2</td>
<td>72.9±1.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Capillary lumen (%)</td>
<td>14.4±0.4</td>
<td>11.4±1.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Endothelial cells (%)</td>
<td>3.4±0.1</td>
<td>3.5±0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Interstitium (%)</td>
<td>4.8±0.4</td>
<td>11.5±3.4</td>
<td>NS</td>
</tr>
<tr>
<td>Interstitial cells (%)</td>
<td>0.62±0.62</td>
<td>0.69±0.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

The volume fraction (%) of myocardial components was measured using a grid consisting of 221 sampling points superimposed on electron micrographs (×5,000, five sections per heart) of left ventricular tissue from aortic stenosis hearts (LVH, n=3 hearts) and sham-operated control hearts (n=3 hearts) (see "Methods"). The volume fraction of myocytes, capillary lumen, endothelial cells, interstitium, and interstitial cells was calculated. Data are expressed as mean±SD.
density is increased throughout the myocardium of hearts with pressure-overload LVH.

We have recently shown that overall, cardiac ACE mRNA levels and activity are increased in rat hearts with pressure-overload LVH.7 In the present investigation, we extend these observations by the finding that ACE density is twofold higher in the myocardium of rats with pressure-overload LVH. Interestingly, the
induction of cardiac ACE is not confined to the epicardial coronary vessels and cardiac valves but is diffusely distributed in the hypertrophied myocardium. The specific cellular sites at which ACE is localized cannot be addressed by this study. In this regard, preliminary immunohistochemical studies demonstrated that peroxidase-labeled goat-anti-rabbit ACE antibodies supplied by Dr. R.I. Soffer, Cornell University Medical College, New York, N.Y.) localized to endothelial cells of myocardial capillaries, arteries, and veins as well as the endocardium. Staining of cardiac myocytes was minimal (J.F.M. Smits, unpublished observations). Furthermore, quantitative morphometric measurements showed a modest increase in fractional myocyte volume and capillary lumen volume without a significant increase in the endothelial cell volume in the hypertrophied hearts. Thus, the twofold increase in ACE density noted above is likely to be related to an upregulation of cardiac ACE expression rather than to a simple increase in the microvascular endothelial cell compartment. The mechanism for the induction of ACE remains unclear.

The functional importance of tissue ACE was demonstrated by intra-arterial infusions of Ang I. More recently, the conversion rate of Ang I was measured biochemically in various tissues, including the heart. In the present study, we confirmed our recent observations that the intracardiac generation of Ang II during perfusion with Ang I was significantly higher in isolated beating hearts with LVH, providing more evidence for an activation of the cardiac converting enzyme in pressure-overload hypertrophy. The key goal of the present study was to test whether a cardiac ACE-specific pathway in LVH accounts for the increased intracardiac Ang I conversion rate and its

| Table 2. Baseline Left Ventricular and Coronary Hemodynamic Parameters |
|---------------------------|-------------------|-----------------|-----------------|------------------|
| CF ([mL/min]/g LV)        | CPP (mm Hg)      | LVP (mm Hg)     | LV dev P/g (mm Hg/g) | LVEDP (mm Hg) |
| LVH 11.7±2.5             | 100±1             | 122±8           | 75±5             | 10±1            |
| Control 13.9±1.0         | 81±1              | 119±10          | 111±8            | 10±1            |
| p NS <0.01               | NS                | NS              | 0.05             | NS              |

Baseline hemodynamics of buffer-perfused, beating control and hypertrophied rat hearts. CF, coronary flow per gram left ventricular weight; CPP, coronary perfusion pressure; LVP, left ventricular pressure; LV dev P/g, left ventricular developed pressure per gram left ventricle; LVEDP, left ventricular end-diastolic pressure. Data are expressed as mean±SEM.

**Figure 4.** Plots showing coronary perfusion pressure (CPP) in left ventricular hypertrophy (LVH) (upper panel) and control hearts (lower panel). Groups of LVH and control hearts were perfused at constant flow rates for 15 minutes with vehicle followed by 30 minutes of angiotensin I (ANG I), perfused for 15 minutes with enalaprilat before and together with 30 minutes of angiotensin I (Enal/ANG I), or perfused for 45 minutes with vehicle only (veh). Angiotensin I increased CPP in both LVH and control hearts. Parallel infusion of enalaprilat significantly diminished angiotensin I effects on CPP.
adverse effects on diastolic function. We have previously shown that enalaprilat may inhibit Ang I conversion in normal rat hearts,7 and pilot studies suggested the possibility of ACE-specific Ang I conversion in the hypertrophied hearts.7 In the present study, enalaprilat reduced the biochemically measured intracardiac Ang I conversion rate by 70% in LVH hearts, suggesting that in this model of pressure-overload hypertrophy, the majority of intracardiac Ang I conversion is catalyzed by ACE or a related enzyme whose function can be inhibited by enalaprilat. Our data are in agreement with studies in normal rat, rabbit, and pig hearts, which demonstrated a dose-dependent decrease of intracardiac Ang I conversion by SQ-20881, captopril, enalaprilat, ramiprilat, and cilazapril,4,7,14–16,50 and studies in normal and cardiomyopathic hamster hearts in which no radiolabeled Ang II was detected in the coronary effluent after perfusion with [125I]-Ang I plus captopril.51

The specific inhibition of cardiac tissue ACE may contribute to cardiac hemodynamic effects of ACE inhibitors.40,52 The Ang I–related decrease of coronary flow in isolated rat hearts perfused at constant coronary perfusion pressure can be prevented by pretreatment with ACE inhibitors.53–55 Accordingly, in the present study in which coronary flow was kept constant, enalaprilat diminished the Ang I–related increase in coronary perfusion pressure and resistance. Cardiac ACE inhibition may also have a favorable electrophysiologic action in the prevention of ventricular arrhythmias during ischemia or reperfusion. Linz et al56 showed that cardiac ACE inhibition with ramiprilat during reperfusion in buffer-perfused rat hearts can protect against ventricular fibrillation and tissue injury during reperfusion. Co-administration of Ang II, but not Ang I, reversed these effects. The positive inotropic effects of Ang II seen in human cardiac muscle in vitro57 appear to be species specific and cannot be studied in the rat or guinea pig at physiological heart rates.7,58 In sheep and cats, cardiac ACE inhibition or Ang II receptor blockade prevented Ang I–related positive inotropy.59 These physiological data in conjunction with data regarding the effects of ACE inhibition on biochemically measured Ang I conversion rate suggest that ACE or an unidentified but functionally closely related enzyme is the major source for converting Ang I to Ang II in the heart. However, not all reports agree that cardiac Ang I effects can be attenuated by ACE inhibition. Hirakata and coworkers51 described positive inotropic effects of Ang I in isolated normal and cardiomyopathic hamster hearts despite the presence of captopril. The conflicting results may be explained by species differences, especially with regard to the expression of other non-ACE Ang I converting enzymes.16–18 Alternatively, the discrepancy may relate to the differences in affinity of the ACE inhibitor to cardiac ACE,60,61 to the selected drug dose, or to the time interval between ACE inhibition.
and onset of Ang I perfusion. In the present investigation, isolated hearts were perfused with $4 \times 10^{-6}$ M enalaprilat 15 minutes before and together with Ang I to allow for complete tissue ACE inhibition.

An additional aim of this study was to examine whether specific cardiac ACE inhibition can affect Ang I conversion and the Ang I–mediated profound depression of diastolic function in hypertrophied hearts. We have previously shown that Ang I perfusion causes a dose-related depression of relaxation and increase in LVEDP. In the present study, we provide evidence that this increase of diastolic pressure in LVH hearts cannot be solely attributed to changes in coronary turgor and pressurization of the coronary vasculature. In particular, coronary flow–related changes of coronary perfusion pressure compatible with the increase of perfusion pressure seen in Ang I perfused directly caused only a minimal increase of LVEDP in LVH hearts. Instead, the effects of Ang II on diastolic function may be mediated by activation of phospholipase C and the generation of phosphoinositide second messengers, which modify the mobilization and reuptake of cytosolic calcium and myofilament calcium sensitivity. In this regard, there is evidence Ang II delays relaxation and slows relaxation velocity in isolated rat myocytes. In addition, there is recent evidence that Ang II potentiates delayed relaxation in isolated hypertrophied myocytes from spontaneously hypertensive rats, consistent with an effect of angiotensin on calcium-mediated force in diastole. In rat hearts with pressure-overload hypertrophy, enalaprilat prevented the Ang I–related depression of diastolic function such that LVEDP was not significantly different from vehicle-perfused time controls. Interestingly, studies in our laboratory by Eberli et al. recently demonstrated in the same model of pressure-overload LVH that enalaprilat can attenuate diastolic dysfunction during low-flow ischemia in hypertrophied hearts as well.

In addition to effects mediated by local Ang II formation, ACE may also interfere with the degradation of the vasoactive peptide bradykinin. The implications of amplified tissue ACE in hypertrophied rat hearts on

**FIGURE 6.** Plots showing isovolumic left ventricular end-diastolic pressure (LVEDP) in left ventricular hypertrophy (LVH) (upper panel) and control hearts (lower panel). Groups of LVH and control hearts were perfused at constant flow rates for 15 minutes with vehicle followed by 30 minutes of angiotensin I (ANG I), perfused for 15 minutes with enalaprilat before and together with 30 minutes of angiotensin I (Enal/ANG I), or perfused for 45 minutes with vehicle only (veh). Angiotensin I increased LVEDP in both LVH and control hearts, although the effect was significantly greater in LVH hearts. Parallel infusion of enalaprilat significantly diminished angiotensin I effects such that Enal/ANG I–treated hearts were not significantly different from respective vehicle-perfused time controls (veh).

**FIGURE 7.** Graph showing the effects of increasing levels of coronary perfusion pressure (CPP) by increasing levels of coronary flow to augment coronary turgor on left ventricular end-diastolic pressure (LVEDP) in left ventricular hypertrophy hearts.
bradykinin metabolism as well as nitric oxide and prostaglandin formation will require further investigation. However, we suggest that in the present study the effects of ACE inhibition were mainly mediated by the inhibition of angiotensin's effect. First, neither normal nor LVH hearts displayed any changes in coronary hemodynamics and cardiac function with enalaprilat alone (before Ang I infusion). Second, bradykinin infusion in isovolumic rabbit or rat hearts (Reference 69 and unpublished observations) has no effect on diastolic function.

It is unclear whether cardiac ACE inhibition improves impaired cardiac relaxation in diseased human hearts with pathological cardiac hypertrophy. Intravenous administration of enalaprilat decreases arterial and coronary venous Ang II concentrations to a similar extent, suggesting that both systemic and cardiac Ang II synthesis are affected by ACE inhibition. Oral treatment with the ACE inhibitor cilazapril in patients with hypertension and hypertensive cardiomyopathy resulted in significantly improved diastolic filling of the left ventricle. Furthermore, intravenous enalaprilat administration improved LVEDPs in normotensive patients with myocardial ischemia, especially during exercise. However, only limited data are available on the effects of intracoronary administration of ACE inhibitors (e.g., isolated cardiac ACE inhibition). Foul et al have shown that intracoronary infusion of enalaprilat in patients with LVH and dilated cardiomyopathy caused a decrease in coronary vascular resistance, a slight depression of indices of systolic pump function, and a reduction in elevated LVEDP without a change in end-diastolic volume, suggesting an improved diastolic compliance. New studies by Friedlich et al have shown a beneficial effect of cardiac ACE inhibition by the intracoronary infusion of enalaprilat on left ventricular diastolic properties in patients with severe hypertrophy due to aortic stenosis in the absence of systemic neurohumoral activation.

Thus, future studies are needed to test the hypotheses that cardiac ACE-dependent Ang II production is present in humans and may contribute to abnormal diastolic function and development of congestive heart failure as well as modulating the development of cardiac hypertrophy in patients with chronic pressure overload from aortic stenosis or hypertension.

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