Increased rat cardiac angiotensin converting enzyme activity and mRNA expression in pressure overload left ventricular hypertrophy. Effects on coronary resistance, contractility, and relaxation.

H Schunkert, … , C S Apstein, B H Lorell

J Clin Invest. 1990;86(6):1913-1920. https://doi.org/10.1172/JCI114924.

Research Article

We compared the activity and physiologic effects of cardiac angiotensin converting enzyme (ACE) using isovolumic hearts from male Wistar rats with left ventricular hypertrophy due to chronic experimental aortic stenosis and from control rats. In response to the infusion of 3.5 X 10(-8) M angiotensin I in the isolated buffer perfused beating hearts, the intracardiac fractional conversion to angiotensin II was higher in the hypertrophied hearts compared with the controls (17.3 +/- 4.1% vs 6.8 +/- 1.3%, P less than 0.01). ACE activity was also significantly increased in the free wall, septum, and apex of the hypertrophied left ventricle, whereas ACE activity from the nonhypertrophied right ventricle of the aortic stenosis rats was not different from that of the control rats. Northern blot analyses of poly(A)+ purified RNA demonstrated the expression of ACE mRNA, which was increased fourfold in left ventricular tissue obtained from the hearts with left ventricular hypertrophy compared with the controls. In both groups, the intracardiac conversion of angiotensin I to angiotensin II caused a comparable dose-dependent increase in coronary resistance. In the control hearts, angiotensin II activation had no significant effect on systolic or diastolic function; however, it was associated with a dose-dependent depression of left ventricular diastolic relaxation in the hypertrophied hearts. These novel observations suggest that cardiac ACE is induced in hearts with left […]

Increased Rat Cardiac Angiotensin Converting Enzyme Activity and mRNA Expression in Pressure Overload Left Ventricular Hypertrophy

Effects on Coronary Resistance, Contractility, and Relaxation

Heribert Schunkert,* Victor J. Dzau,* Shiow Shih Tang,* Alan T. Hirsch,* Carl S. Apstein,‡ and Beverly H. Lorell§

**Molecular and Cellular Laboratory, Division of Vascular Medicine, Brigham and Women's Hospital and Harvard Medical School; ‡the Cardiac Muscle Research Laboratory, the Cardiovascular Institute of Boston University School of Medicine, the Cardiology Section of the Thorndike Memorial Laboratory, Boston City Hospital; §Charles A. Dana Research Institute and the Harvard-Thorndike Laboratory of Beth Israel Hospital, and the Department of Medicine (Cardiovascular Division), Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts 02215*

Abstract

We compared the activity and physiologic effects of cardiac angiotensin converting enzyme (ACE) using isovolumic hearts from male Wistar rats with left ventricular hypertrophy due to chronic experimental aortic stenosis and from control rats. In response to the infusion of 3.5×10^{-8} M angiotensin I in the isolated buffer perfused beating hearts, the intracardiac fractional conversion to angiotensin II was higher in the hypertrophied hearts compared with the controls (17.3±4.1% vs 6.8±1.3%, $P < 0.01$). ACE activity was also significantly increased in the free wall, septum, and apex of the hypertrophied left ventricle, whereas ACE activity from the nonhypertrophied right ventricle of the aortic stenosis rats was not different from that of the control rats. Northern blot analyses of poly(A)$^+$ purified RNA demonstrated the expression of ACE mRNA, which was increased fourfold in left ventricular tissue obtained from the hearts with left ventricular hypertrophy compared with the controls. In both groups, the intracardiac conversion of angiotensin I to angiotensin II caused a comparable dose-dependent increase in coronary resistance. In the control hearts, angiotensin II activation had no significant effect on systolic or diastolic function; however, it was associated with a dose-dependent depression of left ventricular diastolic relaxation in the hypertrophied hearts. These novel observations suggest that cardiac ACE is induced in hearts with left ventricular hypertrophy, and that the resultant intracardiac activation of angiotensin II may have differential effects on myocardial relaxation in hypertrophied hearts relative to controls. (*J. Clin. Invest.* 1990. 86:1913–1920.) Key words: diastole • calcium • aortic stenosis • angiotensin I • angiotensin II

Introduction

There is increasing recognition of the existence of an endogenous renin-angiotensin system in the heart. Evidence supporting the presence of this system in cardiac tissue includes the demonstration of angiotensinogen and renin mRNAs (1–4), and the biochemical identification of renin, angiotensin converting enzyme (ACE),[1] and angiotensin II, as well as its receptor, in the heart (5–9). Furthermore, the intracardiac activation of angiotensin I to angiotensin II has been demonstrated in isolated perfused hearts (10). The physiologic roles of this angiotensin II–generating pathway have not been defined and may include local effects on cardiac contractility, coronary vasomotor tone, arrythmogenesis, as well as a permissive or regulatory role in modulating cardiac growth and development (5, 10). There is indirect evidence that supports the role of the renin-angiotensin system in the initiation of protooncogene expression and cell growth in smooth muscle and myocardial cells (11–17), and inhibition has been shown to prevent left ventricular hypertrophy in rats with pressure overload (18–20), and promote the regression of chronic pressure overload hypertrophy in humans (21, 22). The potential functional significance of the intracardiac conversion of angiotensin I to angiotensin II in the presence of chronic pressure overload hypertrophy is unknown. If angiotensin plays a regulatory role in the development of compensatory pressure overload hypertrophy, the activity of a physiological pathway for the conversion of angiotensin I to angiotensin II may be amplified in hearts with compensatory pressure overload hypertrophy. To test this hypothesis, we compared the conversion of angiotensin I to II and its physiologic effects on coronary vasoreactivity and systolic and diastolic function in isolated beating buffer-perfused hearts from rats with chronic aortic stenosis and from age-matched controls. In addition, ACE activity and mRNA were measured in cardiac tissue obtained from the hypertrophied and control hearts. Our experiments demonstrated that cardiac ACE activity and mRNA were increased in the hypertrophied ventricle and that the increased rate of angiotensin II production was associated with altered diastolic properties in the hypertrophied hearts.

Methods

Preparation of animals. Male Wistar rats were obtained by the Charles River Breeding Laboratories (Wilmington, DE). Aortic stenosis was created in weanling rats (body weight, 100 g; age, 3–4 wk) 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 were subsequently fed normal rat chow (Purina) and water ad libitum, and were used for experimentation 8–9 wk after operation. The body weight was recorded, and serum was obtained from the animals before they were killed.

Address correspondence and reprint requests to Dr. Beverly H. Lorell, Cardiology Division, Beth Israel Hospital, 330 Brookline Ave., Boston, MA 02215, and Dr. Victor J. Dzau, Division of Cardiovascular Medicine, Stanford University School of Medicine, 300 Pasteur Drive, Stanford, CA 94305.

Received for publication 29 March 1990 and in revised form 6 July 1990.

J. Clin. Invest.

0021-9738/90/12/1913/08 $2.00
Volume 86, December 1990, 1913–1920

1. *Abbreviations used in this paper:* ACE, angiotensin converting enzyme; LVEDP, left ventricular enddiastolic pressure; LVH, left ventricular hypertrophy.

Perfusion technique. The isolated isovolumic working rat heart preparation has previously been described in detail (23). Rats were injected intraperitoneally with 1.0–1.5 ml sodium pentobarbital (15 mg/ml) and the thorax was rapidly opened. Within 20 s, the hearts were placed in a water-jacketed constant temperature chamber (37°C) and the coronary arteries were perfused by a constant flow pump (Masterflex; Cole-Parmer Instrument Co., Chicago, IL) 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 (all mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.0, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, lactate 1.0, and glucose 5.5. The perfusate was equilibrated with a 5% CO_2/95% O_2 gas mixture which achieved a PO_2 of ~ 550 mmHg and pH of 7.35–7.40.

A small cannula was inserted into the left ventricular apex to vent any Thebesian drainage. A cannula was inserted into the ligated pulmonary artery 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 vena 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 in the left ventricle and left ventricular pressure was measured via a Statham P23Db pressure transducer (Statham Instruments, Inc., Puerto Rico) connected to the balloon via a short length of stiff polyethylene tubing. The damping characteristics and the natural resonant frequency response of this system (24) satisfy the range shown by Falsetti et al. (25) 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 mmHg in both groups, and this balloon volume was held constant so that an increase in left ventricular end diastolic pressure (LVEDP) signified a decrease in diastolic chamber distensibility (23, 24). Coronary perfusion pressure was measured from a sidearm of the aortic perfusion cannula connected to a Statham P23Db pressure transducer. The coronary flow rate was measured by timed samples of coronary venous effluent collected from the pulmonary artery cannula.

Experimental protocol: infusion of angiotensin I in the isolated perfused hearts. Before each experiment, the heart was perfused for a stabilization period of 20 min at a paced heart rate of 4 Hz which was continued throughout the experiment. In hearts from the rats with chronic aortic stenosis (LVH group, $n = 9$), coronary flow was adjusted to achieve a mean coronary perfusion pressure (CPP) of 100 mmHg and was then fixed at that level of flow throughout the subsequent experiment. In the control group (C, $n = 12$) coronary flow was adjusted to achieve a CPP of 80 mm Hg and was then 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 pilot 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 extraction (26) in both groups. At the end of the stabilization period, measurements of left ventricular pressure and its first derivative, coronary perfusion pressure, and coronary flow were made. Samples of the coronary venous effluent were collected for determination of angiotensin I and angiotensin II content. After baseline measurements, both groups were perfused with buffer containing angiotensin I. Angiotensin I (Sigma Chemical Co., St. Louis, MO) in 0.5% pasteurized BSA (Sigma Chemical Co.) was added to the system at the level of the aortic cannula by a Harvard Pump (flow rate 0.5 ml/min; Harvard Apparatus Co., Natick, MA), to achieve a final concentration of 3.5×10^{-8} M. During the final 5 min of the angiotensin 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. The hearts were then infused for an additional 15 min with angiotensin I at a final concentration of 3.5×10^{-7} M. Measurements of left ventricular function were recorded at the end of the first and second infusion period. Assessment of left ventricular function in response to both concentrations of angiotensin I was done in six hearts each from the LVH and control groups. After each experiment, the left and right ventricles were quickly dissected, weighed, and frozen in liquid nitrogen, and stored at −70°C for subsequent biochemical assay of tissue ACE activity. The atria were discarded since they were ligated during the perfusion period. In four hearts (two from each group), after the initial 15-min infusion of angiotensin I at a concentration of 3.5×10^{-8} M, angiotensin I was infused with the parallel infusion of 5×10^{-7} M enalaprilat, an ACE inhibitor, for 15 min, and the coronary venous effluent was collected during the final 5 min of this infusion period (10).

Biochemical analysis of angiotensin I to 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, MA) as previously described (27). After washing with 0.01 M trifluoroacetic acid, the SEP PAK cartridges were eluted with 80% acetonitrile in 0.01 M trifluoroacetic acid. The eluate was lyophilized, then redissolved in 0.02 M acetic acid and subjected to HPLC. Reverse-phase HPLC was done using a Varian 5000 solvent delivery system combined with a Spectroflow 757 variable UV monitor (LKB Instruments, Paramus, NY) 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 ultropac 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 min. The flow rate was 1 ml/min. The fractions were collected in polypropylene test tubes with a collection time per fraction of 30 s. Synthetic angiotensins were used for calibration of the HPLC column. The recovery was 83% for angiotensin I and 94% for angiotensin II. The fractions corresponding to the retention times of the synthetic angiotensin I, II, and III were pooled. After lyophilization, samples were redissolved in 0.2 ml of 0.02 M acetic acid diluted with RIA buffer (10 mM Tris, pH 7.4 with 1 mg/ml BSA), and RIA performed as previously described (27). The sensitivity of this assay is 0.1–1.2 ng/tube. Since RIA was always performed after HPLC, the crossreactivity of the angiotensin II antibody with angiotensin III or nonangiotensin peptides was avoided. The fractional conversion rate was calculated as [(Ang II, M)/(Ang I + Ang II, M)] × 100.

Measurement of serum and cardiac tissue ACE activity. Serum and cardiac ACE activity was measured by the rate of generation of His-Leu from Hip-His-Leu substrate using fluorometric assay described by Cushman and Cheung (28). Tissue was homogenized in ice-cold 50 mM potassium phosphate buffer, pH 7.5. An aliquot of the homogenized samples was then incubated with 12.5 mM Hip-His-Leu for 10 min at 37°C in a shaking waterbath. The reaction was stopped by adding 280 mM NaOH. Blank controls were treated in the same fashion, with the exception that Hip-His-Leu was added after NaOH treatment. Phthalaldehyde 1% was then added to the aliquots for 10 min before the reaction was stopped with 3 N HCl. The fluorescence at 486 nm was measured using an excitation wavelength of 364 nm (MPF-66 Fluorescence Spectrophotometer; Perkin-Elmer Corp., Norwalk, CT). The fluorescence of phthalaldehyde His-Leu was linear from 0.02 to 12 nmol/min.

Analysis of cardiac ACE mRNA. Four hearts from the LVH and the control groups were removed under anesthesia as described above. Right and left ventricles were quickly dissected, weighed, and snap frozen in liquid nitrogen. The tissue was then homogenized in 4 M guanidine thiocyanate, 0.5% sodium-*n*-lauryl sarcosine, 25 mM sodium citrate, 0.1 M β-mercaptoethanol, and 2 M CsCl. The homogenate was applied over a cushion of autoclaved 5.7 M CsCl, and the RNA was pelleted by centrifugation at 35,000 rpm (8.4×10^4 *g*) for 16 h at 25°C in a Ti 70.1 fixed angle rotor (Beckman Instruments, Palo Alto, CA). The RNA pellets were resuspended in 0.2 M sodium ace-

tate, pH 5.5, gently rocked at 4°C for 1 h, and precipitated in two volumes of ethanol. The precipitated RNA was dissolved in H_2O and the amount of RNA was quantitated by absorbance at 260 nm in duplicate. Poly(A)$^+$ purification was carried out by an oligo(dT)-cellulose column (Collaborative Research, Inc., Waltham, MA) using a method previously described in detail (29). To serve as a recovery marker, 20 μg of anglerfish islet cell RNA was added to each sample before the purification.

For Northern blot analysis, aliquots of poly(A)$^+$ RNA were lyophilized and denatured. The denatured RNA was then size-separated by electrophoresis in 1.5% agarose gel containing 20 mM MOPS, 5 mM sodium acetate, 1 mM EDTA, (pH 7.0), 0.66 M formaldehyde, and 2 μg/ml ethidium bromide. After electrophoresis at 100 V for 4 h, the gels were photographed under ultraviolet (UV) light. The efficiency of the transfer was confirmed by ethidium bromide stain and was examined before and after transfer. The gels were then transblotted to nylon filters (Gene Screen; New England Nuclear, Boston, MA) by capillary action with 10× SSC for 16 h, after which they were cross-linked by exposure to UV light. A plasmid vector (Bluescript KS; pB35-19) containing 3,334 bp of human ACE cDNA was cut with Eco RI and Bgl II to yield 1.7- and 1.6-kb inserts of ACE cDNA. Both fragments were separated from the 3.0-kb vector on an agarose gel and oligolabeled with [^{32}P]dCTP and were then used as probes for ACE mRNA. A solution of 50% formamide, 5× Denhardt's solution, 25 μg/ml yeast tRNA, 25 μg/ml salmon sperm DNA, poly(A)RNA 10 μg/ml, poly(C)RNA 10 μg/ml in 0.2% SDS was used for prehybridizing the blots for 4 h. The blots were then hybridized overnight in the same buffer to which α-^{32}P cDNA was added. After hybridization, the blots were washed in 0.2× SSC with 0.1% SDS at room temperature for 10 min and then three times at 58°C for 30 min. The blots were then exposed to x-ray film (Kodak XAR; Eastman Kodak, Rochester, NY) for 48 h. The autoradiographs were scanned using a microdensitometer (LKB Instruments). Standard RNA samples from adult male rat lungs, testes, and liver (25 μg samples) were run on each Northern blot as interblot reference standards.

Statistical analysis. All values are expressed as the mean±standard error of the mean. Statistical analysis of the effects of angiotensin I infusion on the LVH and control group was done using analysis of variance for repeated measurements and subsequent use of paired *t*-tests. The statistical analysis of differences observed between the LVH and control groups in regard to fractional conversion of angiotensin I to angiotensin II, cardiac tissue ACE activity, and laser densitometry quantification of the ACE mRNA signals was done using Student's *t* test for unpaired data. Statistical significance was accepted at the level of $P < 0.05$.

Results

Extent of hypertrophy. The LVH group had moderate left ventricular hypertrophy relative to the control group with the mean left ventricular wet weight increased 30% above control (0.85±0.03 vs. 0.65±0.04 g, $P < 0.002$). The average body weight was not different in the LVH and control groups (P = NS), and the mean left ventricular-to-body weight ratio was increased 40% above the control group (3.8±0.2 vs. 2.7±0.1 g/kg, $P < 0.005$). The average relative weights of the right ventricles were similar in both groups (0.79±0.08 vs. 0.74±0.04 g/kg, P = NS) (Fig. 1).

Fractional conversion of angiotensin I to II in the isolated perfused heart. Neither of the angiotensins was found in the coronary venous effluent when hearts were perfused with buffer solution alone. In response to perfusion with 3.5×10^{-8} M angiotensin I, the intracardiac conversion rate of angiotensin I to II in the perfused hearts was 17.3±4.1% in the LVH group and 6.8±1.3% in the control group ($P < 0.01$). When the conversion rate was normalized per gram of wet weight cardiac

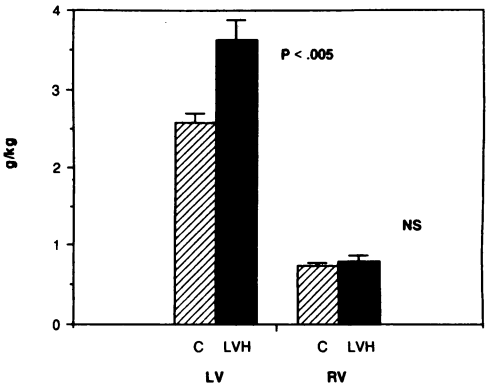

Figure 1. Bar graphs showing the weights of the left ventricle and right ventricle relative to body weight for the LVH group with chronic aortic stenosis and the control group of age-matched and sham-operated rats.

tissue (right and left ventricular weights combined), the fractional conversion rate of angiotensin I to II in the perfused hearts was also higher in the LVH vs. control group (16.2±4.2 vs. 7.9±1.6%, $P < 0.05$). In two hearts from each group, the fractional conversion of angiotensin I was assessed after the addition of enalaprilat (5×10^{-7} M) to the perfusate containing angiotensin I. In the presence of enalaprilat, the conversion rate dropped to 2.9±1% in the LVH group and 2.1±.9% in the control group, documenting that the conversion of angiotensin I to II was related to intracardiac generation of angiotensin II by a specific ACE.

Left ventricular and coronary hemodynamic parameters at baseline are shown in Table I. By study design, coronary flow was adjusted at baseline to achieve a similar coronary flow rate per gram left ventricular weight in the LVH and control groups (Table I). At these levels of myocardial perfusion per gram, coronary vascular resistance was similar. At baseline, at a physiologic paced heart rate of 4 Hz and at an identical left ventricular and diastolic pressure, left ventricular systolic pressure, +dP/dt and developed pressure per unit of left ventricular mass were higher in the LVH group than in the control group (Table I).

In response to the infusion of angiotensin I, there was a dose-related increase in coronary vascular resistance in both the LVH and the control group ($P < 0.05$ for both groups). The increase in coronary vascular resistance was similar for the LVH and control groups (Fig. 2). There was no significant dose-related effect of angiotensin I infusion on left ventricular developed pressure in either the LVH or the control group (Figure 3). There was also no significant dose-related effect of angiotensin I infusion on left ventricular +dP/dt in either group.

Fig. 4 shows the dose-related effects of angiotensin I infusion on isovolumic LVEDP in both groups. At baseline, LVEDP was adjusted to a similar level of 10 mm Hg in the LVH and control groups (Table I). In response to angiotensin I infusion, there was no change in isovolumic LVEDP in the control group. However, the LVH group showed a dose-related increase in LVEDP to a level of 17±2 mm Hg ($P < 0.05$), at a concentration of 3.5×10^{-8} M, and to a level of 30±4 mm Hg ($P = 0.05$) at the higher concentration of 3.5×10^{-7} M. Left ventricular −dP/dt also decreased in the LVH group in comparison with the control group ($P < 0.05$).

Table I. Baseline Left Ventricular and Coronary Hemodynamic Parameters

	LV developed pressure	+dP/dt	LVEDP	−dP/dt	CPP	CF	CVR
	mmHg/g	*mmHg*	*mmHg/s*	*mmHg/s*	*mmHg*	*(ml/min)/g*	*mmHg/(ml/min)/g*
LVH Group	152±6	5,562±307	11.6±0.9	4,540±325	96±4	17.7±2.5	6.1±0.9
Control Group	113±13	2,925±252	10.3±1.0	2,280±201	82±3	14.8±1.5	5.8±0.6
P	<0.05	<0.001	NS	<0.001	<0.05	NS	NS

LV developed pressure, left ventricular developed pressure per gram left ventricular wet weight; +dP/dt, peak positive left ventricular dP/dt; LVEDP, left ventricular end-diastolic pressure; −dP/dt, peak negative left ventricular dP/dt; CPP, coronary perfusion pressure; CF, coronary flow per gram left ventricular wet weight; CVR, coronary vascular resistance.

Measurement of cardiac ACE activity. We studied ACE activity in tissue obtained from three regions of the left ventricle (free wall, septum, and apex) and the right ventricle of the LVH and the control groups. As shown in Fig. 5, in the LVH group, cardiac ACE activity was significantly increased in all three regions of the left ventricle. However, ACE activity was similar in the serum, and in right ventricular tissue from the LVH and the control groups it was similar (24 vs. 27 nm/min/g, P = NS).

Measurement of cardiac ACE mRNA. Northern blot analyses demonstrated ACE mRNA expression in the left ventricles of both groups (Fig. 6). The electrophoretic migration of cardiac ACE mRNA was identical with rat pulmonary ACE mRNA. Rat testicular RNA, an additional control, contained a smaller ACE transcript, similar to that which has already been shown for human and mouse testicular ACE mRNA (30, 31). In left ventricular tissue from the LVH group, cardiac ACE mRNA showed much stronger signals than in sham-operated controls (Fig. 6). Anglerfish insulin mRNA (840 bases), which was added to total RNA before poly(A)$^+$ RNA purification and served as a recovery marker, was well separated from ACE mRNA. Using laser densitometry, we determined the ratios of the signal density for ACE mRNA and anglerfish-insulin mRNA. As shown in Fig. 7, there was a fourfold increase in ACE mRNA in the left ventricular tissue from the LVH group as compared with the controls ($P < 0.05$).

Discussion

Recent observations indicate that endogenous renin-angiotensin systems are localized in multiple tissues, including the heart (1, 5, 32). Biochemical studies have demonstrated the presence of renin and angiotensins (10, 33), and ACE activity in cardiac tissue (28). The presence of high affinity angiotensin II receptors have been demonstrated in the rat conduction system, cardiac endothelium and isolated myocytes (7, 9), and in cardiac tissue from other species (6, 8, 34). The capability for the local synthesis of this system's components is supported by the demonstration of the expression of renin and angiotensinogen mRNAs in rat and mouse hearts (1–4). However, the physiologic significance of the *intracardiac* conversion of angiotensin I to II in the presence of *chronic* pressure overload

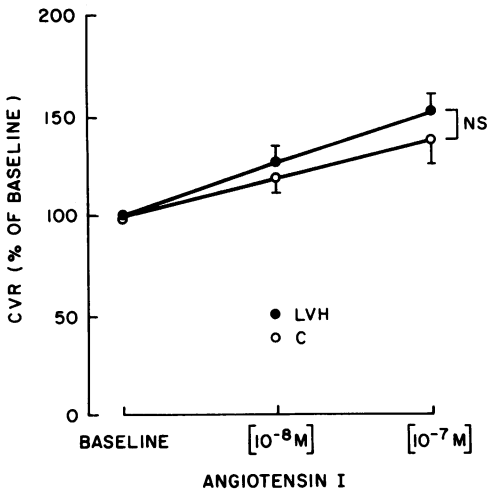

Figure 2. Change in coronary vascular resistance relative to baseline in response to angiotensin I infusion in the LVH and control groups. At baseline, calculated coronary vascular resistance was similar in the LVH and control groups (6.1±0.9 vs. 5.8±0.6 mmHg/(ml/min)/g, P = NS). The intracardiac infusion of angiotensin I resulted in a significant dose-related increase in coronary vascular resistance in both groups; however, there was no difference in the increase in coronary vascular resistance relative to baseline between the LVH and control groups.

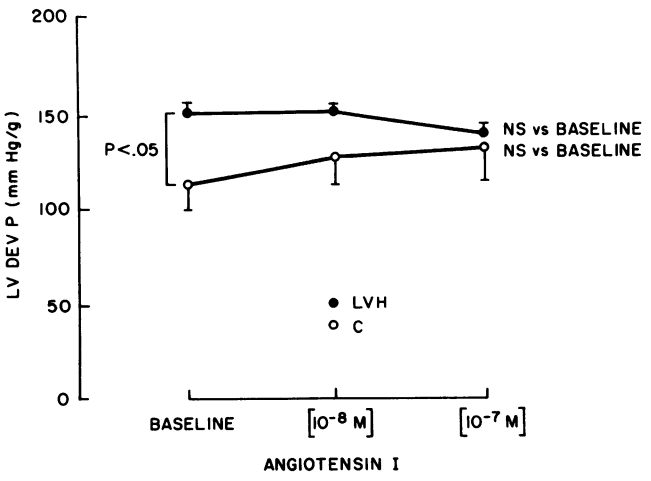

Figure 3. Left ventricular developed pressure per gram left ventricular weight at baseline and in response to angiotensin I infusion in the LVH and control groups. At baseline, left ventricular developed pressure per gram was higher in the LVH vs. control group. In response to angiotensin I infusion, there was no significant increase in left ventricular developed pressure in the LVH or control groups.

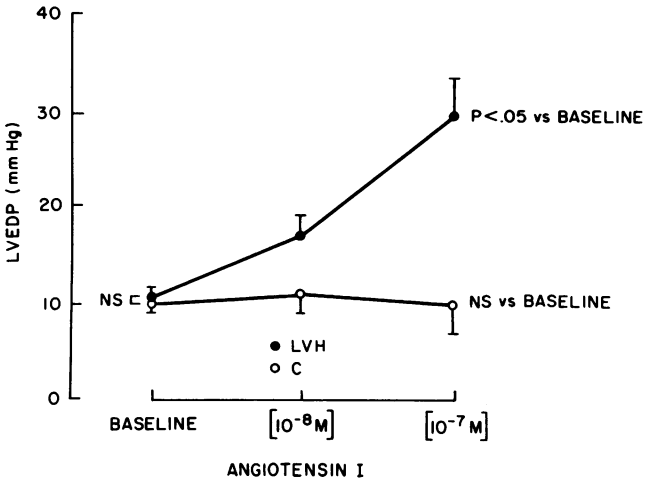

Figure 4. LVEDP at baseline and in response to angiotensin I infusion in the LVH and control groups. There was no change in LVEDP in the control group in response to angiotensin I infusion. However, in response to angiotensin I infusion, there was a marked dose-related increase in isovolumic left ventricular end-diastolic pressure in the LVH group.

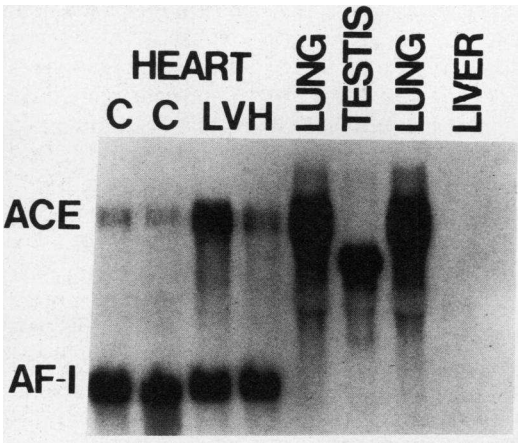

Figure 6. Northern blot analysis of rat testicular and pulmonary ACE mRNA, and cardiac mRNA from left ventricular tissue obtained from the LVH and control groups. The blot was hybridized with an oligolabeled human ACE cDNA. Using 25 mg poly(A)$^+$ RNA, all hearts gave a clear signal. This demonstrates the local expression of ACE mRNA in the rat heart. Furthermore, the signal from tissue of the LVH group hearts is increased relative to the controls. Anglerfish insulin mRNA (AF-I) served as a recovery marker.

hypertrophy is not yet known. To address this issue, we compared the activity and the physiologic effects of ACE in beating isovolumic buffer-perfused hearts from Wistar rats with chronic aortic stenosis and from controls. Our new observations indicate that the intracardiac activation of angiotensin II is increased in the presence of established pressure-overload hypertrophy, and that the activation of this pathway may modify myocardial relaxation in the presence of cardiac hypertrophy.

Intracardiac activation of angiotensin II. The activation of angiotensin II in the heart was first shown by Needleman et al. (35) and was confirmed by Lindpaintner et al. (10), who infused angiotensin I into isolated, buffer-perfused rat hearts and reported a fractional conversion of angiotensin I to II of 6.4% in normal adult rat hearts. Using a similar methodology our findings corroborate this fractional conversion in normal hearts (6.7%). In the studies of Lindpaintner et al. (10, 36), the ACE inhibitors captopril, ramiprilat, and cilaprilat each resulted in an immediate and dose-dependent reduction of angiotensin I to II conversion. In the present study, we used enalaprilat which decreased the intracardiac conversion rate of angiotensin I to II to 2.1%. However, tissue angiotensin II generation might be modulated by an endogenous inhibitor of ACE (37) or angiotensin II–generating enzymes other than ACE, as well (38, 39). Our findings do not exclude the presence of additional angiotensin II–generating enzymes, since enalaprilat inhibited 70%, but not all, of the angiotensin I conversion in normal rat hearts.

The present study was designed to study the role of cardiac ACE in pressure overload hypertrophy. The fractional conversion of angiotensin I to II was significantly higher in hypertrophied hearts (17.3%) as compared with controls, and enalaprilat decreased this conversion rate to 2.9%.

This observation was corroborated by biochemical measurements of ACE activity that showed significantly higher

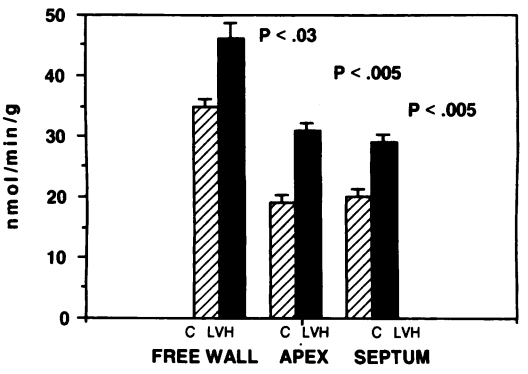

Figure 5. Bar graphs of cardiac ACE activity in tissue from the left ventricular free wall, apex, and septum in the LVH and control groups. Tissue ACE activity was significantly higher in all regions of the left ventricle in the LVH group compared with the control group. However, there was no difference in tissue ACE activity of the nonhypertrophied right ventricle between the LVH group and the control group.

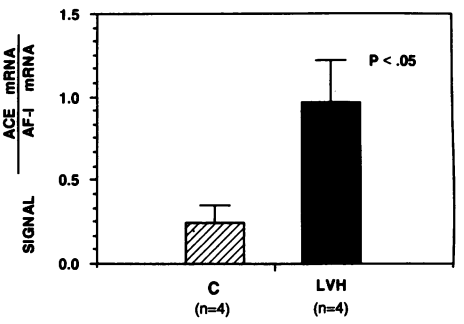

Figure 7. Bar graphs showing quantification by laser densitometry of the signals from the Northern blot analysis of cardiac ACE mRNA in the LVH and control groups. Anglerfish insulin mRNA (AF-I) was used as a recovery marker. ACE mRNA was increased fourfold in left ventricular tissue from the LVH group compared with the control group.

ACE activity in all three regions of the hypertrophied left ventricles relative to the control hearts. In contrast, the ACE activity from the right ventricles, which did not undergo hypertrophy, was not increased relative to that of right ventricular tissue from the control hearts. This shows that the stimulus for increased ACE expression is associated directly with the hypertrophic response to an increase in load, and is not a systemic or blood-borne factor. This is consistent with the finding of Hirsch et al. (40) of a regional increase in tissue ACE activity in noninfarcted tissue that had undergone compensatory hypertrophy in rats after coronary ligation relative to sham-operated controls. Recent studies have demonstrated multiple sites of ACE localization, including the coronary vasculature, atrial and ventricular myocardium, and the valve leaflets in the rat heart (41), but we have not yet localized the primary sites of the intracardiac ACE in this aortic stenosis rat model.

Induction of cardiac ACE mRNA. In this study, we showed for the first time that ACE messenger RNA is locally expressed in the heart, and that its expression is induced more than fourfold in left ventricular tissue from hypertrophied hearts relative to controls. It remains to be established whether the enhanced expression of ACE is consistent with the reinduction of a fetal pattern of gene expression in chronic hypertrophy (42). Increased angiotensin II synthesis may play a role in the initiation of cardiac hypertrophy. There is evidence that angiotensin II can stimulate protooncogene induction, protein synthesis, and cell growth in smooth muscle and myocardial cells (12–17, 43, 44).

Our observation of increased expression of ACE mRNA in the hypertrophied left ventricles of rats with chronic aortic stenosis suggests that the cardiac angiotensin system may also contribute to the maintenance of *established* pressure overload hypertrophy. This notion is supported by the observation that chronic converting enzyme inhibition caused regression of hypertrophy in the model of experimental aortic stenosis that we used in our study (18), and by the preliminary finding that left ventricular angiotensinogen mRNA levels are several-fold higher in spontaneously hypertensive rats with chronic hypertrophy compared with nonhypertrophied controls (45).

Physiologic effects of angiotensin II activation. Our study also showed that the intracardiac conversion of angiotensin I to II causes dynamic changes in cardiac physiology. The infusion of angiotensin I elicited a dose-related increase in coronary vascular tone in the isolated hearts that was of similar magnitude in the LVH and control groups. The finding that the relative change in coronary vascular resistance was similar in both groups in the presence of an increased conversion of angiotensin I to II in the hypertrophied hearts raises the possibility that the effect of angiotensin II on coronary vascular reactivity is blunted in the hypertrophied heart. In this regard, preliminary studies on angiotensin II receptor density and affinity in hypertrophied left ventricular tissue from rats with aortic stenosis demonstrated a lower receptor density and no change in affinity compared with sham-operated control hearts (Tang, S. S., personal communication). Alternatively, the site of increased production of angiotensin II in the hypertrophied heart may be at the level of the myocyte rather than the vasculature.

Heart rate was controlled by pacing, and we did not study the chronotropic effects of angiotensin II. Angiotensin II activation did not elicit a statistically significant dose-dependent positive inotropic effect in either the LVH or the control group. Conflicting observations of the inotropic effects of angiotensin II may be related to experimental differences in the confounding factor of angiotensin-induced sympathetic neurotransmitter release (46), and variation between species. Positive inotropic effects, independent of the β-adrenergic system and cyclic AMP activation, have been reported for the rabbit, cat, dog, and hamster (6, 39, 47–50). In contrast, no response or a negative inotropic response has been reported for the rat and guinea pig (10, 34, 51) over a concentration range of angiotensin II similar to that obtained by the fractional conversion of angiotensin I in our study.

The direct myocardial effects of angiotensin II activation on diastolic relaxation have received little attention. In this study, angiotensin II activation had no effects on isovolumic left ventricular diastolic pressure in the control group. In contrast, angiotensin I infusion caused a significant dose-dependent increase in isovolumic left ventricular diastolic pressure in the LVH group consistent with a decrease in diastolic distensibility (23, 24). In isovolumic buffer-perfused hearts, consideration must be given to the contribution of coronary turgor on changes in diastolic pressure (52). However, in this experiment, changes in coronary turgor are unlikely to account for the deterioration of diastolic function in the LVH group since coronary flow was held constant and was similar in both groups at baseline and during angiotensin I infusion.

Our findings are consistent with recent observations of Allen et al. (51) using isolated beating rat myocytes who found that angiotensin II increased the "L-type" Ca^{2+} current in the absence of any effect on adenylate cyclase, whereas both shortening and relaxation velocity decreased. These findings lend support to the notion that the effects of angiotensin in our isolated rat heart model were direct rather than being mediated by sympathetic neurotransmitter release (46). Instead, the effects of angiotensin II on cardiac performance (34, 51, 53, 54) and on growth (16, 17) appear to be associated with the activation of phosphoinositide second messengers (55–57) and changes in the mobilization and reuptake of cytosolic $[Ca^{2+}]_i$. Cardiac $[Ca^{2+}]_i$ homeostasis, which is a major determinant of diastolic function, is profoundly altered in hypertrophied rat myocytes (57–59), and there is evidence that IP_3-induced sarcoplasmic reticulum Ca^{2+} release is enhanced (60). We postulate that the deterioration of diastolic function induced by angiotensin II activation that we observed may be related to the effects of phosphoinositide second messengers on the slowed $[Ca^{2+}]_i$ reuptake which is characteristic of hypertrophied myocytes.

Additional studies will need to be done to determine if our findings are relevant to pressure-overload in other species and in humans with chronic cardiac hypertrophy. In this regard, Foult et al. have shown that the intracoronary infusion of the ACE inhibitor enalaprilat in patients with increased left ventricular mass and dilated cardiomyopathy causes a fall in coronary vascular resistance, a slight depression of indices of systolic pump function, and a reduction in elevated left ventricular diastolic pressure without change in end-diastolic volume (61). These data are consistent with the hypothesis that the intracardiac activation of angiotensin II is present in humans, and may contribute to abnormal diastolic function and development of congestive heart failure in patients with chronic hypertrophy from aortic stenosis and hypertension.

Acknowledgments

We acknowledge the assistance of Chris E. Talsness in the performance of the fluorimetric analyses. We appreciate the assistance of William N. Grice in performing the perfusion studies and statistical analysis and the surgical assistance of Souen Ngoy in the preparation of the aortic stenosis colony. We gratefully acknowledge Dr. P. Corvol and Dr. F. Soubrier for providing the human ACE cDNA, and Dr. A. Freedlander for providing the angiotensin II antibody. We also appreciate the assistance of William Cook and Kathleen Whelan in preparation of the manuscript.

This work was supported in part by grants from the National Heart, Lung, and Blood Institute (HL-28939, B. H. Lorell; HL-31807 and HL-38189, C. S. Apstein; HL-35610, HL-35792, HL-19259, HL-35252, HL-40210, HL-4266, HL-36568, V. J. Dzau, HL-43131, S. S. Tang), an Established Investigatorship of the American Heart Association (B. H. Lorell), and a research grant from the Deutsche Forschungsgemeinschaft (H. Schunkert, Schu 672/1-1).

References

1. Jin, M., J. W. Markus, R. E. Lang, T. Unger, K. Lindpaintner, and D. Ganten. 1988. Endogenous tissue renin-angiotensin systems. *Am. J. Med.* 84:28–36.

2. Dzau, V. J., K. E. Ellison, T. Brody, J. Ingelfinger, and R. E. Pratt. 1987. A comparative study of the distributions of renin and angiotensin messenger ribonucleic acids in rat and mouse tissues. *Endocrinology.* 120:2334–2338.

3. Ohkubu, H., K. Nakayama, T. Tanaka, and S. Nakanishi. 1986. Tissue distribution of rat angiotensinogen mRNA and structural analysis of its heterogeneity. *J. Biol. Chem.* 261:319–323.

4. Kanapuli, S. P., and A. Kumar. 1987. Molecular cloning of human angiotensinogen cDNA and evidence for the presence of its mRNA in rat heart. *Circ. Res.* 60:786–790.

5. Dzau, V. J. 1988. Cardiac renin-angiotensin system. Molecular and functional aspects. *Am. J. Med.* 84:22–27.

6. Baker, K. M., C. P. Campanile, G. J. Trachte, and M. J. Peach. 1984. Identification and characterization of the rabbit angiotensin II myocardial receptor. *Circ. Res.* 54:286–293.

7. Rogers, T. B., S. H. Gaa, and I. S. Allen. 1986. Identification and characterization of functional angiotensin II receptors on cultured heart myocytes. *J. Pharmacol. Exp. Ther.* 236:438–444.

8. Wright, G. B., R. W. Alexander, L. S. Ekstein, and M. A. Gimbrone. 1983. Characterization of the rabbit ventricular myocardial receptor for angiotensin II. *Mol. Pharmacol.* 24:213–221.

9. Saito, K., J. S. Gutkind, and J. M. Saavedra. 1987. Angiotensin II binding sites in the conduction system of rat hearts. *Am. J. Physiol.* 253 (*Heart Circ. Physiol.* 22):H1618–H1622.

10. Lindpaintner, K., M. Jin, M. J. Wilhelm, F. Suzuki, W. Linz, B. A. Schoelkens, and D. Ganten. 1988. Intracardiac generation of angiotensin and its physiologic role. *Circulation* 77(Suppl I):1–18.

11. Naftilan, A. J., R. E. Pratt, C. S. Eldridge, H. L. Lin, and V. J. Dzau. 1989. Angiotensin II induces c-fos expression in smooth muscle via transcriptional control. *Hypertension.* 13:706–711.

12. Naftilan, A. J., R. E. Pratt, and V. J. Dzau. 1989. Induction of platelet-derived growth factor A chain and c-myc gene expressions by angiotensin II in cultured rat vascular smooth muscle cells. *J. Clin. Invest.* 83:1419–1424.

13. Geisterfer, A. A. T., M. J. Peach, and G. K. Owens. 1988. Angiotensin II induces hypertrophy, not hyperplasia, of cultured rat aortic smooth muscle cells. *Circ. Res.* 62:749–756.

14. Robertson, A. L., and P. A. Khairallah. 1971. Angiotensin: rapid localization in nuclei of smooth and cardiac muscle. *Science (Wash. DC).* 172:1138–1140.

15. Khairallah, P. A., and J. Kanabus. 1983. Angiotensin and myocardial protein synthesis. *Perspect. Cardiovasc. Res.* 8:337–347.

16. Hori, M., K. Iwai, K. Iwakara, H. Sato, and A. Kitabatake. 1989. Angiotensin II stimulates protein synthesis in neonatal rat cardiomyocytes through enhanced Na^+/H^+ exchange (abstract). *Circulation.* 80(Suppl. II):II–450.

17. Katoh, Y., I. Komuro, Y. Shibasaki, H. Yamaguchi, and Y. Yazaki. 1989. Angiotensin II induces hypertrophy and oncogene expression in cultured rat heart myocytes (abstract). *Circulation.* 80(Suppl II):II–450.

18. Kromer, E. P., and G. A. J. Riegger. 1988. Effects of long-term angiotensin converting enzyme inhibition on myocardial hypertrophy in experimental aortic stenosis in the rat. *Am. J. Cardiol.* 62:161–163.

19. Sen, S. 1983. Regression of cardiac hypertrophy: experimental animal model. *Am. J. Med.* 75(Suppl. 3A):87–93.

20. Pfeffer, J. M., and M. A. Pfeffer. 1988. Angiotensin converting enzyme inhibition and ventricular remodeling in heart failure. *Am. J. Med.* 84(Suppl. 3A):37–44.

21. Wakashima, Y., F. M. Foued, and R. C. Tarazi. 1984. Regression of left ventricular hypertrophy from systemic hypertension by enalapril. *Am. J. Cardiol.* 53:1044–1049.

22. Devereaux, R. B., T. G. Pickering, R. J. Cody, and J. H. Laragh. 1987. Relation of renin-angiotensin system activity to left ventricular hypertrophy and function in experimental and human hypertension. *J. Clin. Hypertens.* 3:87–103.

23. Lorell, B. H., L. F. Wexler, S. Momomura, E. Weinberg, and C. S. Apstein. 1986. The influence of pressure overload left ventricular hypertrophy on diastolic properties during hypoxia in isovolumically contracting rat hearts. *Circ. Res.* 58:653–663.

24. Serizawa, T., W. M. Vogel, C. S. Apstein, and W. Grossman. 1981. Comparison of acute alterations in left ventricular diastolic chamber stiffness induced by hypoxia and ischemia. *J. Clin. Invest.* 68:91–102.

25. Falsetti, H. J., R. E. Mates, R. J. Carroll, R. L. Gupta, and A. C. Bell. 1974. Analysis and correction of fluid wave distention in fluid filled catheter systems. *Circulation.* 49:165–172.

26. Apstein, C. S., E. Puchner, and N. Brachfeld. 1970. Improved automated lactate method. *Anal. Biochem.* 38:20–34.

27. Kifor, I., and V. J. Dzau. 1987. Endothelial renin-angiotensin pathway: evidence for intracellular synthesis and secretion of angiotensins. *Circ. Res.* 60:422–428.

28. Cushman, D. W., and H. S. Cheung. 1971. Concentrations of angiotensin-converting enzyme in tissues of the rat. *Biochem. Biophys. Acta.* 250:261–265.

29. Aviv, H., and P. Leder. 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. *Proc. Natl. Acad. Sci. USA.* 69:1408–1412.

30. Soubrier, F., F. Alhenc-Gelas, C. Hubert, J. Allegrini, J., M. John, G. Tregear, and P. Corvol. 1988. Two putative active centers in human angiotensin I converting enzyme revealed by molecular cloning. *Proc. Natl. Acad. Sci. USA.* 85:9386–9390.

31. Bernstein, K. E., B. M. Martin, A. S. Edwards, and E. A. Bernstein. 1989. Mouse angiotensin-converting enzyme is a protein composed of two homologous domains. *J. Biol. Chem.* 264:11945–11951.

32. Dzau, V. J. 1987. Implications of local angiotensin production in cardiovascular physiology and pharmacology. *Am. J. Cardiol.* 59:59A–65A.

33. Dzau, V. J., and R. N. Re. 1987. Evidence for the existence of renin in the heart. Circulation. 75(Suppl. I):134–136.

34. Baker, K. M., and H. A. Singer. 1988. Identification and characterization of guinea pig angiotensin II ventricular and atrial receptors: coupling to inositol phosphate production. *Circ. Res.* 62:896–904.

35. Needleman, P., G. R. Marshall, and B. E. Sobel. 1975. Hormone interactions in the isolated rabbit heart. *Circ. Res.* 37:802–808.

36. Lindpaintner, K., M. J. Wilhelm, M. Jin, T. Unger, R. E. Lang, B. A. Schoelkens, and D. Ganten. 1987. Tissue renin-angiotensin systems: focus on the heart. *J. Hypertens.* 5(Suppl. II):S33–S38.

37. Ikemoto, F., G-B Song, M. Tominaga, and K. Yamamoto. 1989. Endogenous inhibitor of angiotensin converting enzyme in the rat heart. *Biochem. Biophys. Res. Commun.* 159:1093–1099.

38. Urata, H., B. Healy, R. W. Stewart, F. M. Bumpus, and A. Husain. 1990. Angiotensin II-forming pathways in normal and failing human hearts. *Circ. Res.* 66:883–890.

39. Hirakata, H., F. Fouad-Tarazi, F. M. Bumpus, M. Khosla, B. Healy, A. Husain, H. Urata, and H. Kumagai. 1990. Angiotensins and the failing heart. Enhanced positive inotropic response to angiotensin I in cardiomyopathic hamster heart in the presence of captopril. *Circ. Res.* 66:891–899.

40. Hirsch, A. T., C. Talsness, A. Lage, and V. J. Dzau. 1989. The effect of experimental myocardial infarction and chronic captopril treatment on plasma and tissue angiotensin enzyme activity (abstract). *Clin. Res.* 37(Suppl. A):266A.

41. Johnston, C. I., B. Fabris, H. Yamada, A. O. Frederick, F. A. O. Mendelsohn, R. Cubela, D. Sivell, and B. Jackson. 1989. Comparative studies of tissue inhibition by angiotensin converting enzyme inhibitors. *J. Hypertension* 7(Suppl. 5):SH–S16.

42. Izumo, S., B. Nadal-Ginard, and V. Mahdavi. 1988. Protooncogene induction and reprogramming of cardiac gene expression produced by pressure overload. *Proc. Natl. Acad. Sci. USA.* 85:339–343.

43. Re, R. N., D. L. Vizard, J. Brown, and S. E. Bryan. 1984. Angiotensin II receptors in chromatin fragments generated by micrococcal nuclease. *Biochem. Biophys. Res. Commun.* 119:220–227.

44. Jackson, T. R., L. A. C. Blair, J. Marshall, M. Goedert, and M. R. Hanley. 1988. The *mas* oncogene encodes an angiotensin receptor. *Nature (Lond.).* 335:437–440.

45. Li, C., O. Prakash, and R. N. Re. 1989. Altered regulation of angiotensin gene in the left ventricles of the hypertensive rats (abstract). *Circulation.* 80(Suppl. II):II–450.

46. Xiang, J., W. Linz, H. Becker, D. Ganten, R. E. Lang, B. Scholkens, and T. H. Unger. 1985. Effects of converting enzyme inhibitors: ramipril and enalapril on peptide action and sympathetic neurotransmission in the isolated heart. *Eur. J. Pharmacol.* 113:215–223.

47. Freer, R. J., A. J. Pappano, M. Peach, K. Bing, M. McLean, S. Vogel, and N. Sperelakis. 1976. Mechanism of the positive inotropic effect of angiotensin II on isolated cardiac muscle. *Circ. Res.* 39:178–183.

48. Koch-Weser, J. 1964. Myocardial actions of angiotensin. *Circ. Res.* 14:337–344.

49. Dempsey, P. J., Z. T. McCallum, K. M. Kent, and T. Cooper. 1971. Direct myocardial effects of angiotensin II. *Am. J. Physiol.* 220:477–481.

50. Kobayashi, M., Y. Furukawa, and S. Chiba. 1978. Positive chronotropic and inotropic effects of angiotensin II in the dog heart. *Eur. J. Pharmacol.* 50:17–25.

51. Allen, I. S., N. M. Cohen, R. S. Dhallen, S. T. Gaa, W. J. Lederer, and T. B. Rogers. 1988. Angiotensin II increases spontaneous contractile frequency and stimulates calcium current in cultured neonatal rat heart myocytes: insights into the underlying mechanisms. *Circ. Res.* 62:524–534.

52. Vogel, W. M., C. S. Apstein, L. L. Briggs, W. H. Gaasch, and J. Ahn. 1982. Acute alterations in left ventricular diastolic chamber stiffness: Role of the "erectile" effect of coronary arterial pressure and flow in normal and damaged hearts. *Circ. Res.* 51:465–478.

53. Dosemeci, A., R. Dhallen, N. M. Cohen, W. J. Lederer, and T. B. Rogers. 1988. Phorbol ester increases calcium current and simulates the effects of angiotensin II on cultured neonatal rat heart myocytes. *Circ. Res.* 62:347–357.

54. Capogrossi, M. C., T. Kaku, D. G. Pelto, C. Filburn, R. G. Hansford, H. Spurgeon, and E. G. Lakatta. 1987. Phorbol ester translocates protein kinase C and has a negative inotropic effect in rat cardiac myocytes. *Biophys. J.* 51:112a. (Abstr.)

55. Nosek, T. M., M. F. Williams, S. T. Aeigler, and R. E. Godt. 1986. Inosital triphosphate enhances calcium release in skinned cardiac and skeletal muscle. *Am. J. Physiol.* 250(*Cell. Physiol.* 19):C807–C811.

56. Massey, C., W. J. Lederer, and T. B. Rogers. 1989. Phorbol esters depress sarcoplasmic reticulum function in permeabilized cultured neonatal rat myocytes (Abstract). *Circulation.* 80(Suppl. II):II–446.

57. Keung, E. C. 1989. Calcium current is increased in adult myocytes from hypertrophied rat myocardium. *Circ. Res.* 64:753–761.

58. De la Bastie, D., D. Levitsky, L. Rappaport, J. J. Mercardier, F. Marotte, C. Wisnewsky, V. Brovkovich, K. Schwartz, and A.-M. Lompre. 1990. Function of the sarcoplasmic reticulum and expression of its Ca^{2+}-ATPase gene in pressure overload-induced cardiac hypertrophy in the rat. *Circ. Res.* 66:554–564.

59. Hanf, R., I. Drubaix, and L. Lelievre. 1988. Rat cardiac hypertrophy: altered sodium-calcium exchange in sarcolemmal vesicles. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 236:145–149.

60. Kawaguchi, H., M. Shouki, and H. Yasuda. 1989. Calcium release from microsomes was stimulated by inositol triphosphate in rat heart (abstract). *Circulation.* 80(Suppl. II):II–443.

61. Foult, J.-M., O. Tavolaro, I. Antony, and A. Nitenberg. 1988. Direct myocardial and coronary effects of enalaprilat in patients with dilated cardiomyopathy: assessment by a bilateral intracoronary infusion technique. *Circulation.* 77:337–344.