Effect of Angiotensin Converting Enzyme Inhibition on Pressure-Induced Left Ventricular Hypertrophy in Rats

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The influence of angiotensin converting enzyme inhibition on the development of left ventricular (LV) hypertrophy due to stenosis of the aortic arch was studied in female Sprague-Dawley rats. The aortic arch was banded to an outer diameter of 1.0 mm. After 14 days, LV and right ventricular functional parameters and transstenotic pressure gradient were measured in anesthetized rats. In addition, regional heart weights were determined, and myocytes of three different heart regions were isolated and subjected to morphometric analysis. To inhibit the angiotensin converting enzyme, ramipril was administered orally by gavage in a single daily dose of 1 mg/kg. Rats with aortic stenosis showed a marked increase in LV systolic pressure, mean prestenotic aortic pressure, and LV stroke work compared with sham-operated rats and demonstrated a systolic transstenotic pressure gradient of 82 mm Hg. This increase in LV hemodynamic load was paralleled by the development of LV hypertrophy as determined by a 37% increase in LV weight and by a 20% increase in cell volume of isolated LV myocytes. Concomitant ramipril treatment did not significantly affect LV functional parameters. The transstenotic pressure gradient was the same as in untreated rats with aortic stenosis. Likewise, the weight gain of the LV as well as the development of cellular hypertrophy of the LV were not influenced. Thus, in this model, angiotensin converting enzyme inhibition did not reduce the development of LV hypertrophy independent of the hemodynamic load. (Circulation Research 1991;69:609–617)

Left ventricular (LV) hypertrophy is an independent risk factor for a number of severe cardiac dysfunctions.1,2 The identification of factors that interfere with the development of LV hypertrophy is therefore of considerable clinical significance. The mechanical load imposed on the LV is an important determinant of myocardial structure. This has been shown in many experimental studies, in both in vivo and in vitro preparations.3–5 On the other hand, clinical and experimental observations suggest that the degree of cardiac hypertrophy is not only load dependent.6–9

Among other factors, the renin-angiotensin system (RAS) has emerged as a serious candidate in mediating cardiac myocyte growth for several reasons. Long considered to be a basically humoral hormone system, this view has been extended by the demonstration of local RAS in several tissues including the heart.10 Furthermore, functional angiotensin (Ang) II receptors have been shown to exist on bovine ventricular sarcolemma and on cultured cardiac myocytes.11,12 Finally, Ang II seems to play an important role in the regulation of cell growth and protein synthesis in aortic smooth muscle cells13–16 and cultured cardiac myocytes.16–19 Indeed, clinical and experimental studies have demonstrated regression of LV hypertrophy after angiotensin converting enzyme (ACE) inhibition.20,21

Since ACE inhibitors reduce total peripheral resistance and blood pressure, it is difficult or impossible to decide whether the regression of LV hypertrophy is the result of the decreased afterload or a direct effect on the myocardium mediated by growth control. To examine this question in vivo, an experimental model with fixed hemodynamic afterload, such as the aortic stenosis model, is required. However, the models applied so far in small rodents have some
disadvantages. A stenosis placed immediately on the supravalvular aorta makes it difficult to measure reliably the LV parameters or the transstenotic pressure gradient. Stenosis of the abdominal aorta leaves a considerable part of the circulation as a reactive vascular bed. Thus, in both cases it is difficult to determine the exact hemodynamic load imposed on the LV and to be sure that treated and untreated animals are subjected to a similar pathophysiological condition. Therefore, we have developed a rat model that allows an exact characterization of LV load by LV catheterization and by determination of the transstenotic pressure gradient.

The present study shows that in this model ACE inhibition with ramipril has no major influence on the development of LV hypertrophy when the mechanical load imposed on the LV is similar in treated and untreated animals.

Materials and Methods

The experiments were done on female Sprague-Dawley rats of about 200–240 g body weight at the beginning of the study. The animals were divided into four main groups: two groups of sham-operated rats (sham groups), one without and one with ramipril treatment, and two groups of rats with aortic stenosis (AS groups), one without and one with concomitant ramipril treatment. Ramipril was dissolved in distilled water and administered orally in a single daily dose of 1 mg/kg body wt over the entire period of the study (14 days). The treatment was started 1 hour before the surgical procedure. The rats were anesthetized (100 mg/kg i.p. thiopental sodium) 24 hours after the last administration, and hemodynamic parameters were obtained using ultraminiature catheter pressure transducers (models PR 249 and SPR 392, Millar Instruments, Houston). After these measurements, the hearts were rapidly excised. In one series of experiments, the aorta was cannulated, and the hearts were perfused with collagenase for isolation of cardiac myocytes. In another series of experiments, the hearts were used for the determination of wet and dry weights and for the measurements of LV RNA and DNA concentration and glucose-6-phosphate dehydrogenase (G-6-PDH) activity.

Surgical Procedure

In ether-anesthetized, ventilated rats, the chest was opened by a midsternal incision, and the aortic arch was exposed. The stenosis was produced using a wire of 1.0-mm diameter, which was placed alongside the aortic arch and tightly fixed with a thread around the aortic arch. The wire was removed leaving the aortic arch constricted to an outer diameter equivalent to the diameter of the wire. The constriction was positioned between the branching point of the common carotid arteries (Figure 1, left panel). This enabled us to measure LV parameters and blood pressure in the ascending aorta by catheterization via the right carotid artery and to obtain poststenotic pressure in the descending aorta by catheterization via the left carotid artery (Figure 1, right panel). Sham-operated rats underwent an identical procedure except that the thread was fixed. The chest was closed, and the rats recovered rapidly. The average weight gain of the four groups during the following 2 weeks was not significantly different.

Hemodynamic Measurements

Hemodynamic parameters (LV systolic pressure, LV end-diastolic pressure, LV dP/dt max, prestenotic and poststenotic aortic pressure, right ventricular [RV] systolic pressure, and RV dP/dt max) were measured using Millar ultraminiature catheter pressure transducers. The determination of poststenotic pressure required the additional catheterization of
the left carotid artery. This consistently caused a small increase in pressure of 10–15 mm Hg throughout all groups. Thus, it did not affect comparisons between the groups; however, the control values appeared to be slightly higher than usual. Heart rate, $dP/dt_{\text{max}}$, and pressure were recorded simultaneously on a Brush 2600 recorder (Gould, Cleveland, Ohio). Cardiac output was determined using the thermodilution technique. One hundred microliters of 0.9% NaCl cooled to 18°C was injected into the right atrium. The thermodilution curve was obtained by a thermosensitive implantable microprobe (1.5F, Columbus Instruments, Columbus, Ohio) placed in the ascending aorta. Cardiac output was calculated by a Cardiomax II computer (Columbus Instruments).

Total peripheral resistance index was calculated by dividing mean pressure of the ascending aorta by cardiac output. The transstenotic pressure gradient was obtained by comparing prestenotic and poststenotic pressures, which were recorded simultaneously. Stroke volume was calculated by dividing cardiac output by heart rate; the stroke work index was calculated by multiplying prestenotic mean aortic pressure and stroke volume.

**Isolation of Myocytes and Determination of Cell Size**

After the functional measurements, the hearts were quickly removed, and the aorta was cannulated for retrograde coronary artery perfusion with calcium-free Joklik media containing collagenase. The hearts were divided into RV free wall, septum, and LV free wall by sharp dissection after the collagenase treatment for 18 minutes. The tissue was minced in calcium-free media, and isolated cells were poured through a nylon mesh (250 μm) into a fixation solution. The final concentration of the fixative was 1.5% glutaraldehyde in 0.08 M phosphate buffer. Isolated myocytes were centrifuged through 4% Ficoll in 0.15 M phosphate buffer. This procedure removed most capillaries, blood cells, and debris. Rod-shaped cells with normal sarcomere structure and no visible membrane damage were classified as undamaged. Each region of all preparations characteristically contained more than 70% undamaged cells. A channelizer (model 256, Coulter Corp., Hialeah, Fla.) was used to determine the volume of fixed, isolated myocytes. The Coulter system determines cell volume by measuring the changes in electrical resistance due to displacement of electrolyte as cells move through an aperture. The shape factor (1.05) used in this study was taken from Hurley. Cell length of undamaged myocytes was determined directly by using a microscope equipped with phase optics. Mean cross-sectional area was obtained by dividing cell volume by cell length. Compared with histometric techniques, the Coulter channelizer system gives reliable values for the size of myocytes. Since it was not possible to determine cell size and the other parameters (regional heart weight, percentage dry weight, G-6-PDH activity, and RNA/DNA concentrations) in the same rat, two subgroups were formed out of each main group. The functional parameters (i.e., transstenotic pressure gradient in AS rats) did not differ between the respective subgroups.

**Heart Weights and Measurement of Metabolic Parameters**

After excision of the heart, the RV free wall was trimmed away, and the ventricles were weighed. The LV was divided into three portions and stored under liquid nitrogen until the determination of G-6-PDH and RNA/DNA concentrations.

**G-6-PDH.** The tissue was placed into ice-cold KCl solution (0.15 M containing 0.02 M KHCO₃) and homogenized. Centrifugation, dialysis of the supernatants, and measurement of enzyme activity were done according to the methods of Gloc and McLean. Protein concentration in the dialysate was determined using the biuret reaction. The enzyme activity was expressed as units per gram protein.

**RNA/DNA tissue concentrations.** RNA and DNA tissue concentrations of the LV were determined photometrically according to a modified Schmidt-Thannhauser procedure. Briefly, the frozen tissue samples were ground to a fine powder under liquid nitrogen and extracted with 0.3N perchloric acid. After centrifugation, the sediments were washed three times with 0.3N perchloric acid, and the lipids were extracted. RNA was hydrolyzed by incubation of the samples in 0.5N KOH for 14 hours at room temperature. After addition of 0.5 ml of 6N perchloric acid, the probes were kept in an ice bath for 1 hour. The precipitate (containing the DNA) was separated by centrifugation, and the supernatant (containing the RNA) was decanted. For estimation of DNA, the sediments were suspended in 2 ml of 1N perchloric acid and incubated at 70°C for 1 hour. To eliminate errors arising from the release of proteins mainly in the RNA fraction during the alkaline digestion in 0.5N KOH, the supernatants were measured at two wavelengths (RNA, 260 and 286 nm; DNA, 268 and 284 nm). Using myocardial tissue enriched with calf thymus DNA (Sigma Chemical Co., St. Louis) and calf liver RNA (Sigma), we found that the RNA concentration was slightly overestimated by about 10%, whereas the DNA concentration was determined correctly. Since the overestimation was of comparable extent in all determinations, comparison of the different groups was possible.

**Determination of Plasma ACE Activity**

After the hemodynamic measurements had been completed, blood was taken from the jugular vein for the determination of the plasma ACE activity (24 hours after the last ramipril administration). ACE activity was measured using a commercial photometric test kit (Paesel GmbH & Co., Frankfurt, FRG). The test is based on the further development of the measuring principle proposed by Hayakari et al. The enzyme is offered the synthetic substrate hip-pyrull-L-histidyl-L-leucine, the digestion of which is
TABLE 1. Effect of Ramipril on the Remodeling of the Heart Induced by Aortic Constriction as Measured by the Changes in Left and Right Ventricular Parameters in Myocytes

<table>
<thead>
<tr>
<th></th>
<th>LVW (mg)</th>
<th>LVW/BW (mg/g)</th>
<th>RVW (mg)</th>
<th>CL (μm)</th>
<th>CSA (μm²)</th>
<th>CV (μm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>n</td>
<td>Mean</td>
<td>n</td>
<td>Mean</td>
<td>n</td>
</tr>
<tr>
<td>Sham</td>
<td>544±18</td>
<td>5</td>
<td>2.35±0.02</td>
<td>5</td>
<td>145±8</td>
<td>5</td>
</tr>
<tr>
<td>Sham/R</td>
<td>547±12</td>
<td>5</td>
<td>2.35±0.04</td>
<td>5</td>
<td>141±7</td>
<td>5</td>
</tr>
<tr>
<td>AS</td>
<td>722±30*</td>
<td>10</td>
<td>3.22±0.16</td>
<td>10</td>
<td>146±7</td>
<td>10</td>
</tr>
<tr>
<td>AS/R</td>
<td>685±27*</td>
<td>9</td>
<td>3.10±0.14</td>
<td>9</td>
<td>136±5</td>
<td>9</td>
</tr>
<tr>
<td>LV Mean</td>
<td>365±8†</td>
<td>11</td>
<td>225±9*</td>
<td>114±5</td>
<td>145±6</td>
<td>110±5</td>
</tr>
<tr>
<td>LV n</td>
<td>349±5</td>
<td>10</td>
<td>149±10</td>
<td>116±10</td>
<td>109±6</td>
<td>82±7</td>
</tr>
<tr>
<td>LV W</td>
<td>133±2</td>
<td>8</td>
<td>274±10</td>
<td>8</td>
<td>36,621±1,391*</td>
<td>8</td>
</tr>
<tr>
<td>LV TPR</td>
<td>129±7</td>
<td>4</td>
<td>185±7</td>
<td>4</td>
<td>23,849±764</td>
<td>4</td>
</tr>
<tr>
<td>LV CO</td>
<td>126±2</td>
<td>8</td>
<td>186±10</td>
<td>8</td>
<td>23,477±129</td>
<td>8</td>
</tr>
</tbody>
</table>

Values are mean±SEM. LV, left ventricle (or left ventricular); RV, right ventricle (or right ventricular); LVW, LV weight; LVW/BW, LV weight/body weight; RVW, RV weight; CL, cell length; CSA, cross-sectional area; CV, cell volume; n, number of experiments; Sham, sham-operated rats; Sham/R, sham-operated rats with ramipril treatment; AS, rats with aortic stenosis; AS/R, rats with aortic stenosis and concomitant ramipril treatment.

*p<0.05 compared with Sham.

Results

The different groups exhibited similar mean weight gain during the 2 weeks after surgery. None of the AS rats showed pleural or abdominal effusions. The percent dry weight of the LV was not significantly different between the groups.

RV Parameters

RV functional parameters (RV pressure and RV dP/dt max) were essentially the same throughout all four groups (data not shown). Likewise, RV weight as well as RV myocyte dimensions did not show significant differences (Table 1). Both AS groups exhibited a reduction in the RV/LV weight ratio, demonstrating the development of isolated LV hypertrophy.

LV Functional Parameters

There were no major differences in LV functional parameters between treated and untreated sham-operated rats (Tables 2 and 3). LV systolic pressure, dP/dt max, systolic and diastolic aortic pressure, and peripheral resistance tended to be reduced in ramipril-treated rats; however, these differences were not significant (Tables 2 and 3). Both sham groups exhibited no detectable pressure gradient in the aortic arch.

Constriction of the aortic arch resulted in a marked elevation of LV systolic pressure, prestenotic aortic pressure, and stroke work index (Tables 2 and 3). In addition, LV end-diastolic pressure was significantly enhanced. However, contractility as measured by LV dP/dt max and cardiac output were not altered compared with sham-operated rats (Tables 2 and 3). Poststenotic aortic pressure was in the same range as the aortic pressure determined in sham-operated rats. Thus, a transstenotic systolic pressure gradient of 82 mm Hg had developed in these rats (Table 2).

Concomitant treatment with ramipril did not result in a substantial change of LV functional parameters (Figure 2, Tables 2 and 3). LV systolic pressure, transstenotic pressure gradient, and stroke work index were almost the same in both AS groups. The

TABLE 2. Influence of Aortic Constriction Without and With Concomitant Ramipril Treatment on Functional Parameters

<table>
<thead>
<tr>
<th>Aortic pressure (mm Hg)</th>
<th>HR (beats/min)</th>
<th>Prestenotic</th>
<th>Poststenotic</th>
<th>TPG (mm Hg)</th>
<th>CO (ml/kg · min)</th>
<th>TPRI (ml Hg · kg · min/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham (n=11)</td>
<td>360±12</td>
<td>157±8</td>
<td>121±7</td>
<td>351±18</td>
<td>0.41±0.04</td>
</tr>
<tr>
<td></td>
<td>Sham/R (n=10)</td>
<td>349±5</td>
<td>149±10</td>
<td>116±10</td>
<td>375±19</td>
<td>0.37±0.05</td>
</tr>
<tr>
<td></td>
<td>AS (n=18)</td>
<td>332±8</td>
<td>231±7*</td>
<td>115±5</td>
<td>82±7</td>
<td>357±9</td>
</tr>
<tr>
<td></td>
<td>AS/R (n=17)</td>
<td>365±8†</td>
<td>225±9*</td>
<td>114±5</td>
<td>80±6</td>
<td>373±12</td>
</tr>
</tbody>
</table>

Values are mean±SEM. HR, heart rate; TPG, systolic transstenotic pressure gradient; CO, cardiac output; TPRI, total peripheral resistance index; n, number of experiments; Sham, sham-operated rats; Sham/R, sham-operated rats with ramipril treatment; AS, rats with aortic stenosis; AS/R, rats with aortic stenosis and concomitant ramipril treatment.

*p<0.05 compared with Sham; †p<0.05 compared with AS.
TABLE 3. Changes in Left Ventricular Parameters Induced by Aortic Constriction Without and With Concomitant Treatment With Ramipril

<table>
<thead>
<tr>
<th></th>
<th>LVSP (mm Hg)</th>
<th>dP/dt max (mm Hg/sec)</th>
<th>LVEDP (mm Hg)</th>
<th>SV (ml/kg)</th>
<th>SWI (mm Hg · ml/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham (n=11)</td>
<td>157±8</td>
<td>9,709±695</td>
<td>4±1</td>
<td>1.00±0.07</td>
<td>137±10</td>
</tr>
<tr>
<td>Sham/R (n=10)</td>
<td>149±10</td>
<td>8,433±419</td>
<td>5±1</td>
<td>1.12±0.05</td>
<td>139±7</td>
</tr>
<tr>
<td>AS (n=18)</td>
<td>231±7*</td>
<td>9,894±411</td>
<td>10±1*</td>
<td>1.07±0.03</td>
<td>185±6*</td>
</tr>
<tr>
<td>AS/R (n=17)</td>
<td>225±8*</td>
<td>10,950±631</td>
<td>7±1*</td>
<td>1.03±0.04</td>
<td>172±6*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; SV, stroke volume; SWI, stroke work index; n, number of experiments; Sham, sham-operated rats; Sham/R, sham-operated rats with ramipril treatment; AS, rats with aortic stenosis; AS/R, rats with aortic stenosis and concomitant ramipril treatment.

*p<0.05 compared with Sham.

differences in heart rate, dP/dt max, and cardiac output were comparatively small and did not exceed 12%.

LV Weight and Cell Size Data

LV weight and LV weight/body weight ratio were similar in sham-operated rats without and with ramipril treatment (Table 1). However, cell length, cross-sectional area, and cell volume were uniformly reduced in ramipril-treated sham-operated rats (Table 1). In AS rats, the increase in LV work load was reflected in altered weight and cell size, indicating a remodeling of the left heart chamber. Weight as well as LV weight/body weight ratio showed a considerable increase (Table 1). Although to a smaller extent, the increase in LV weight was paralleled by an enhancement in cell volume (Table 1). Since cell length was hardly affected (+5%), an increase in cross-sectional area (+12%) was mainly responsible for the larger cell volume. The cell size data obtained from the septum were similar to data from the LV in all groups (data not shown). None of the data concerning LV mass of ramipril-treated AS rats differed significantly compared with untreated AS rats (Figure 3). LV weight and the LV weight/body weight ratio were only insignificantly lower in this group. The cell size data were nearly identical to data of untreated AS rats. Thus, the degree of the LV hypertrophy was hardly affected by the ACE inhibition.

Biochemical Measurements

Plasma ACE activity. Twenty-four hours after the last ramipril administration, plasma ACE activity was suppressed by about 80% in both ramipril-treated groups (Table 4).

RNA/DNA concentrations. Both AS groups exhibited a tendency to an increase in RNA concentration; however, this was not significant. Since DNA concentrations were similar in all groups (data not shown), the RNA/DNA ratio also turned out to be insignificantly higher in both AS groups. Ramipril treatment alone had no effect on RNA concentration or on the RNA/DNA ratio.

G-6-PDH. G-6-PDH activity was slightly elevated in AS rats. Compared with the sham group, the difference was significant only for the untreated AS
group. Administration of ramipril did not affect myocardial G-6-PDH activity.

Discussion

RV Parameters

Since RV functional parameters did not differ among the four groups, it seems likely that the LV pressure overload was in a compensated state. Severe LV dysfunction was shown to affect RV function via remodeling of the pulmonary vascular bed and to be associated with RV hypertrophy due to increased pulmonary artery pressure.28 The absence of pleural or abdominal effusions and the morphometric data of the RV (Table 1) support this conclusion.

LV Parameters

Biochemical changes. RNA concentration and the RNA/DNA ratio were not significantly different between the four groups, although both groups with aortic constriction exhibited slightly higher values. Since RNA concentration is increased during the early rapid phase of development of hypertrophy,29 these data suggest that the initial phase of induction of hypertrophy is over after 2 weeks, and major changes in heart weight are not likely to occur at that time.

No differences in DNA concentration were detected between the four groups. The nuclei of cardiac myocytes account for about 25% of the nuclei found in heart tissue, and cardiac myocytes lose their mitotic capacity shortly after birth.30,31 Consequently, variations in DNA concentration predominantly reflect changes in the nonmyocyte cell compartments of the heart. Thus, it seems that in the present study no major alterations in the proportion of nonmyocardial cells have occurred. Furthermore, the applied pressure overload was not capable of producing extensive proliferation of connective tissue, which has been described for sudden pressure overload.32

G-6-PDH activity was slightly elevated in both AS groups with no significant difference between treated and untreated rats (Table 4). G-6-PDH is the rate-limiting enzyme of the oxidative pentose phosphate pathway; thus, it controls the availability of 5-phosphoribosyl-1-pyrophosphate, which is an essential precursor for the synthesis of cardiac adenine nucleotides. An enhanced synthesis of cardiac adenine nucleotides during the development of experimental cardiac hypertrophy has repeatedly been demonstrated, and the availability of 5-phosphoribosyl-1-pyrophosphate has been recognized as one of the most important factors involved.33 When the small elevation in G-6-PDH activity after 2 weeks is compared with the marked elevation seen during the development of LV hypertrophy,33 it appears that in our model LV hypertrophy is fully established after 2 weeks in both treated and untreated rats. Taken together with the above-mentioned data on RNA and DNA concentration, these results suggest that ACE inhibition did not affect the time course of the development of hypertrophy in this model.

Functional changes. Fourteen days after surgery, the hemodynamic situation of the LV was characterized by a considerable increase in load. LV systolic pressure, LV end-diastolic pressure, mean prestenotic aortic pressure, and stroke work index were significantly increased compared with the values in the sham group. Again, there were no signs of severe impairment of LV function as indicated by normal values for heart rate, cardiac output, stroke volume, and contractility, which were essentially the same as in sham-operated rats. In addition, the elevation of LV end-diastolic pressure was less pronounced than in a state of severe depression in LV function.28

It is crucial for the estimation of the direct effects of ACE inhibition on the heart that treated and untreated animals with aortic constriction exhibit similar hemodynamic loads. This was the case in our study. Although some minor differences existed, the functional parameters determining the load imposed on the LV were essentially the same in both AS groups (Figure 2).

Morphological changes. Although plasma ACE activity was markedly depressed in sham and AS rats with ramipril treatment (Table 4), the development of LV hypertrophy as determined by the LV heart weight/body weight ratio was not significantly affected (Figure 3). This is in contrast to recent reports on LV hypertrophy after aortic constriction.31,34,35 These reports state that ACE inhibition influenced
the development of LV hypertrophy independent of blood pressure control. It has to be emphasized that the studies of Linz et al. and Baker et al. have used a different pathophysiological model for the pressure overload. Hence, their results cannot be directly compared with our data.

Linz et al. performed a constriction of the abdominal aorta above the left renal artery, which might result in a different activation of systemic and/or local RAS. Furthermore, the increase in blood pressure was relatively small, and the time course was different. Interestingly, neither nifedipine nor dihydralazine, in doses capable of reducing blood pressure to an extent similar to that of 1 mg/kg/day ramipril, showed a significant effect on the development of hypertrophy. On the other hand, a small dose of ramipril (10 μg/kg/day), which did not influence the elevated blood pressure, prevented LV hypertrophy. This underlines the pathophysiological significance of the RAS in their model. Baker et al. also applied constriction of the abdominal aorta in rats. The increase in cardiac load in their study was mild, as indicated by a mean carotid artery pressure of ~140 mm Hg. However, the significance of their hemodynamic data is difficult to judge, since carotid pressure was measured only in a small portion of the animals with aortic constriction and since the carotid pressure of the sham animals was lacking. The study made by Kromer and Riegger used a model similar to ours; they constricted the ascending aorta. In accordance with our results, ACE inhibition applied immediately from the onset of the pressure overload was not effective in preventing hypertrophy. On the other hand, delayed onset of ACE inhibition reduced hypertrophy, a result that is difficult to interpret. Unfortunately, they did not provide exact hemodynamic data of treated and untreated animals with aortic constriction. Thus, a direct correlation between hemodynamic load and degree of the hypertrophy is lacking in the data of Kromer and Riegger.

On the other hand, our results are in agreement with recent studies using models of RV hypertrophy. One study used pulmonary artery constriction to increase RV afterload, and no dissociation between hemodynamic load and RV weight was observed in animals with ACE inhibition. Clozel et al. applied the model of hypoxic pulmonary hypertension. ACE inhibition with cilazapril completely prevented the remodeling of the pulmonary arteries, whereas the hypoxia-induced increase in pulmonary artery pressure and the concomitant RV hypertrophy were not significantly affected. Thus, in their model the RAS apparently played no major role in the adaptation of the RV but was very important in vascular remodeling.

Concerning the influence of ACE inhibition on the development of LV hypertrophy, the cell size data confirm the results obtained by the measurement of LV weight. Ramipril did not affect the increase in cell volume after aortic stenosis, nor did it influence the size of the hypertrophied myocytes (Figure 3). The increase in cell volume of both AS groups resulted from an increase in mean cross-sectional area rather than from alterations in cell length. This is a feature characteristic for compensated concentric hypertrophy evoked by pressure overload. The percent increase in cell volume was less pronounced compared with the increase in weight (Figure 3). This provides indirect evidence that the pressure overload in our study does not lead to major cell loss due to focal necrosis, which has been described in severe pressure overload. In this case, one would expect an overproportional increase in cell volume compared with the increase in LV weight, as has been shown for isoproterenol-induced hypertrophy.

By directly comparing the data on weight and cell size one might speculate that aortic stenosis had resulted in an increase in LV myocyte number. We do not draw this conclusion for several reasons. Although myocyte hyperplasia has been described in experimental cardiac hypertrophy, direct evidence such as the demonstration of adult myocytes undergoing mitosis has never been presented. Furthermore, our data are limited by the fact that weight and cell morphology were not determined in the same hearts. The exact reason for the discrepancy between increase in weight and increase in cell volume is not clear but may be related to changes in the nonmyocyte compartment or the known variability in cell size between animals.

In contrast to AS rats, concomitant ramipril treatment diminished cell volume and cross-sectional area in sham rats (Table 1). LV systolic pressure, diastolic aortic pressure, and peripheral resistance were also decreased in this group, though not significantly. Because of the small number of animals, it seems impossible to determine whether this effect occurred independent of work load.

Do our results allow us to draw the conclusion that the RAS is not important in the development of cardiac hypertrophy? We feel that this statement cannot be made and that the data should be interpreted more carefully. Most important in this context is whether the tissue RAS is, in fact, suppressed by ramipril. We showed that plasma ACE activity was markedly diminished by the ramipril treatment. Ung et al. have previously demonstrated comparable inhibition of ACE activity in plasma and cardiac tissue using the same dose of ramipril. Linz et al. have shown a significant decrease in plasma Ang II levels using 1 mg/kg/day ramipril. In addition, they were successful in antagonizing hypertrophy by applying a dose of ramipril 100 times less than the dose used by us, so one might conclude that our dose should be sufficient.

However, it is possible that the situation might change under pathophysiological circumstances. A recently published article by Hirakata et al. showed that Ang I infusion in isolated hamster hearts produced a positive inotropic response despite the presence of captopril and that the positive inotropism to Ang I was accentuated in cardiomyopathic hearts. Since the inotropic response could be antagonized by
an Ang II receptor antagonist, they concluded that the conversion of Ang I was mediated by an alternative converting enzyme. The questionable effect of ramipril on the cell size of sham-operated rats in our study might give rise to the speculation that Ang II formation was influenced only in normal hearts. Urata et al found that in normal and in failing human hearts the major enzymatic pathway for Ang II formation is not blocked by ACE inhibitors, suggesting that cardiac Ang II formation is not abolished during chronic therapy. Since chronic treatment with ACE inhibitors enhances plasma levels of Ang I, local Ang II concentrations in the heart could even be increased although the Ang II–forming pathway of the heart is partially blocked.

The situation is further complicated by findings illustrating that plasma and tissue Ang II levels do not appear to be closely correlated to myocardial hypertrophy. Brilla et al compared LV hypertrophy and collagen accumulation in three models of pressure overload with different profiles of plasma Ang II and aldosterone levels. Hypertrophy was closely correlated to ventricular loading but not to plasma Ang II levels, whereas accumulation of collagen seemed to depend on Ang II and aldosterone levels. Recently, Brilla and Weber presented results suggesting that LV hypertrophy in spontaneously hypertensive rats occurs independent of myocardial Ang II levels. They applied a small dose of lisinopril that did not affect blood pressure but normalized the high tissue Ang II levels. Despite the normal Ang II levels, the hypertrophy was not influenced; however, the fibrosis found in untreated spontaneously hypertensive rats was prevented. Taken together, these results favor an important role of Ang II in the pathogenesis of myocardial fibrosis rather than in the remodeling of the myocyte. One can also speculate that in some models ACE inhibition might act indirectly on myocyte remodeling by changing passive properties of myocardial tissue such as stiffness.

In conclusion, the results of this study demonstrate that in our model of aortic constriction chronic ACE inhibition does not influence the development of the LV hypertrophy independent of cardiac work load. However, this does not rule out the possibility that Ang II is a local growth factor at the cardiac myocyte level. A better understanding of regulation of local RAS and further in vivo investigations using Ang II receptor antagonists are necessary for this determination.

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