The Renin-Angiotensin System and Volume Overload–Induced Changes in Cardiac Collagen and Elastin

Marcel Ruzicka, MD, PhD; Fred W. Keeley, PhD; Frans H.H. Leenen, MD, PhD, FRCPC

Background Besides cardiac load, the renin-angiotensin system (RAS) and aldosterone may regulate collagen accumulation during maturation or hypertrophic growth. The effect of cardiac volume overload on both left ventricular (LV) and right ventricular (RV) collagen and elastin and the possible role of the RAS in such changes have not yet been assessed.

Methods and Results In the present study we assessed (1) the effects of 4 to 10 weeks of volume overload by an aortocaval shunt or minoxidil on LV and RV collagen and elastin and (2) the potential of the angiotensin-converting enzyme inhibitor enalapril and the angiotensin II receptor blocker losartan to prevent and regress volume overload–induced changes in cardiac collagen and elastin. Cardiac volume overload by aortocaval shunt or minoxidil treatment decreased LV collagen accumulation as compared with control rats. In contrast, RV collagen accumulation was potentiated during the initial weeks but not during chronic aortocaval shunt. Enalapril and losartan prevented the relative decreases in LV collagen content and concentration induced by a shunt. Losartan also reversed the decrease in LV collagen content by aortocaval shunt. Neither blocker significantly affected the enhanced RV collagen accumulation during the initial weeks of shunt, but both blockers further potentiated RV collagen accumulation during chronic volume overload. Aortocaval shunt for 4 weeks but not 10 weeks enhanced LV and RV elastin accumulation. This initial increase in LV and RV elastin content was blocked by both enalapril and losartan.

Conclusions Cardiac volume overload, even when accompanied by increased plasma renin activity, decreases LV collagen accumulation, suggesting that in contrast to the stimulatory effect of systolic wall stress, increased diastolic wall stress inhibits collagen accumulation. In support of this concept, enalapril and losartan decreased LV preload and maintained LV collagen accumulation. In contrast to LV collagen, RV collagen accumulation was potentiated during the initial weeks of volume overload, possibly related to acute RV pressure overload shortly after aortocaval shunt and its decrease with chronic shunt. Enalapril and losartan had minimal effect on the enhanced RV collagen during the initial weeks of aortocaval shunt but potentiated RV collagen during chronic shunt, possibly by decreasing RV diastolic pressures. Altogether, these data suggest that during cardiac volume overload, the RAS affects cardiac collagen primarily by its hemodynamic effects. The RAS, however, may potentiate RV and LV elastin accumulation during the initial weeks of volume overload since both enalapril and losartan block this increase. (Circulation. 1994;90:1989-1996.)

Key Words • collagen • elastin • shunts • angiotensin

The hypertrophic response of cardiomyocytes to cardiac pressure overload is frequently accompanied by increased accumulation of fibrillar collagen in the myocardium.1-11 Pressure overload–induced interstitial and perivascular myocardial fibrosis may increase myocardial stiffness leading ultimately to ventricular dysfunction5-8,9 and may increase propensity to arrhythmias.10 Perivascular and interstitial myocardial fibrosis, however, develops only in some models of cardiac pressure overload, suggesting the involvement of nonhemodynamic trophic stimuli in the regulation of connective tissue growth.4,12 Humoral trophic stimuli such as angiotensin II and aldosterone have been implicated in this regard.4,7,8,10-12 Development of myocardial fibrosis in the nonhypertrophied right ventricle during arterial pressure overload further supports this concept.4,12

Whereas changes in the connective tissue in response to cardiac pressure overload have been extensively documented,12 minimal data are available regarding the effect of cardiac volume overload on myocardial composition. Cardiac volume overload by aortocaval shunt for 2 months caused a small decrease in the left ventricle (LV) collagen volume fraction in dogs.13 Similarly, Michel et al4 reported small decreases in LV collagen density after 1 or 3 months of cardiac volume overload by aortocaval shunt in rats. However, changes in right ventricle (RV) connective tissue and plasma renin activity (PRA) were not evaluated in either study.

The possible changes in myocardial composition during chronic volume overload by the arterial vasodilator minoxidil have not yet been evaluated in normotensive rats. The increase in both content and concentration of the left ventricle by minoxidil in spontaneously hypertensive rats15,16 suggests a drug-induced stimulation of connective tissue accumulation. Minoxidil-induced cardiac volume overload per se17,18 and/or increases in cardiac trophic stimuli such as the renin-angiotensin system (RAS)17,19 or sympathetic nervous system20-22 may be involved in its effect on connective tissue.

In the present study, we assessed the changes in both LV and RV myocardial collagen and elastin in relation to changes in cardiac hemodynamics during the development and maintenance of volume overload–induced...
cardiac hypertrophy by aortocaval shunt and by chronic minoxidil treatment. Two models of volume overload were studied to address the general paradigm of cardiac volume overload rather than possible model-specific mechanisms. Increases in both cardiac renin activity and PRA accompany the development of cardiac hypertrophy in these two models. During chronic volume overload, an increase in PRA persists in rats on minoxidil, and, depending on the size of aortocaval shunt, may remain increased in rats with aortocaval shunt.

To assess the possible role of the RAS in the changes of cardiac connective tissue, the effects of two different blockers of the RAS, ie, the angiotensin-converting enzyme (ACE) inhibitor enalapril and the angiotensin II receptor blocker losartan, were evaluated with respect to both prevention and regression. Two different types of blockers were used because non–ACE-generated formation of angiotensin II may occur in the heart, and/or part of the effects of ACE blockade may relate to decreased degradation of bradykinin. Volume overload–induced changes in central and peripheral hemodynamics as well as the effects of enalapril and losartan on these changes were previously reported in detail. However, since both cardiac preload and afterload may be involved in the regulation of growth of cardiac connective tissue, cardiac pressures (ie, LV end-diastolic pressure and LV peak systolic pressure) have been included in the present study.

Methods

Animals

Male Wistar rats (weight, 200 to 225 g; about 10 weeks old) were obtained from Charles River Breeding Laboratories (Montreal, Canada). Rats were housed two per cage in a climatized room (24°C) of the animal care unit, kept on a 12-hour light-dark cycle, and given food (Purina rat chow, 120 μmol Na/g food) and water ad libitum. After an acclimatization period of at least 3 days, the rats were randomized into three groups: control, aortocaval shunt, and minoxidil (120 mg/L of drinking water). An abdominal aortocaval shunt was produced by using an 18-gauge disposable needle (Becton-Dickinson) similar as in our previous studies. Sham-operated animals serving as controls were subjected to the same surgical procedure with the exception of puncturing the aorta and the inferior vena cava. Animals were weighed on the day of randomization, weekly thereafter, and on the day of collection of tissue samples. The following three study protocols were performed (protocols 1 and 2 are schematically outlined in Fig 1).

Time Course of Changes in Cardiac Elastin and Collagen Accompanying Cardiac Volume Overload by Aortocaval Shunt

Changes in LV and RV weights and cardiac elastin and collagen content were evaluated at 1 and 4 weeks (development of cardiac hypertrophy) and 9 to 10 weeks (maintenance phase) after shunt surgery. At each time point of follow-up, seven rats per group were studied.

Effects of Enalapril and Losartan on Changes in Cardiac Elastin and Collagen Induced by Aortocaval Shunt

For prevention purposes, treatment with enalapril (250 mg/L in drinking water) or losartan (40 mg/kg per day by gastric gavage) was started 3 days before shunt or sham shunt surgery and continued for 4 weeks. For the assessment of regression, treatment with the two blockers was started the fifth week after surgery and continued for 5 weeks. At each time point of follow-up, seven rats per group were studied.

Changes in Cardiac Elastin and Collagen by Chronic Minoxidil Treatment

Cardiac elastin and collagen were evaluated after 10 weeks on treatment and compared with control rats. Each experimental group consisted of seven rats.

Central Hemodynamics

At each time point of follow-up, rats were anesthetized with halothane–nitrous oxide–oxygen, and a PE-50 catheter (Clay Adams) filled with heparinized saline (100 U/mL) was inserted into the left ventricle through the right common carotid artery. Catheters were exteriorized on the necks of the animals. Rats were allowed to recover from anesthesia for a period of 4 hours. LV end-diastolic pressure (LVEDP) and LV peak systolic pressure (LVPSP) were assessed in conscious, unrestrained rats after a 30-minute acclimatization period, as previously described.

Cardiac Weight

Under pentobarbital anesthesia, the chest cavity was opened; the heart was arrested in diastole by intravenous injection of 1 mol/L KCl, rapidly excised, and placed into ice-cold saline to remain in diastole and to remove the blood. After removal of the atria and large vessels, the ventricles were blotted dry and the right ventricle was dissected along its septal insertion from the rest of the ventricular mass. Wet LV and RV weights were assessed. Both ventricles were put immediately on dry ice and kept frozen (−70°C) until the collagen and elastin assessment.

Collagen and Elastin Contents

Right ventricles and left ventricles were homogenized separately in 0.9% NaCl using a Polytron homogenizer. Total collagen and elastin contents were measured separately. Because of the relatively small proportion of collagen in ventricular tissue, a hot trichloroacetic acid (TCA) extraction procedure was used to concentrate the collagen before analysis. Three volumes of 5% TCA at 4°C was added to the homogenate, and the insoluble material was collected by centrifugation.

Three volumes of 5% TCA was added to the pellet, and the sample was placed in a water bath at 95°C for 60 minutes, with occasional mixing. Insoluble material was collected by centrifugation, and the pellet was reextracted with hot TCA in the same manner. Extracts were pooled, and 12 mol/L HCl was added to give a final concentration of 5.7 mol/L. HCl. The samples were then hydrolyzed for 24 hours at 100°C. Total hydroxyproline in this hydrolysate was determined by a chemical method based on the technique of Kivirikko et al. Total elastin was determined as the insoluble residue remaining after NaOH extraction. These residues were confirmed to be essentially pure elastin by amino acid analysis. The washed
between groups at a given treatment period were evaluated by ANOVA and Duncan's multiple range test. Differences were considered statistically significant at \( P<.05 \).

**Results**

**Maturation-Related Changes in LV and RV Collagen and Elastin**

From 11 to 20 weeks of age, body weight in control rats increased from 322±11 to 506±13 g (\( P<.05 \)). LV weight increased from 648±24 to 873±33 g (\( P<.05 \)) and RV weight from 146±10 to 193±6 g (\( P<.05 \)). LV and RV collagen content increased by about 60% (\( P<.05 \)) and 100% (\( P<.05 \)), respectively (Fig 2). LV collagen content increased in parallel with the increase in LV weight and thus LV collagen concentration remained relatively stable (Table 1). In contrast, RV collagen accumulation outpaced increases in RV mass, resulting in an increase in collagen concentration (\( P<.05 \); Table 1). LV and RV elastin accumulation increased in parallel with LV and RV weight up to 14 weeks of age. Whereas LV and RV weight continued to increase from 14 to 20 weeks of age, LV and RV elastin content no longer increased (Fig 2), resulting in significant decreases in LV and RV elastin concentration at 20 versus 14 weeks (Table 2).

**Effects of Enalapril and Losartan on Maturation-Related Changes in LV and RV Collagen and Elastin**

Treatment for 4 to 5 weeks with either enalapril or losartan when started at 10 weeks (Fig 3) and to a lesser extent when started at 15 weeks of age (Fig 4)
inhibited LV collagen accumulation. Since both blockers also caused decreases in LV weight, decreases in the LV collagen concentration were less pronounced (Table 1).

In contrast, enalapril and losartan increased RV collagen content and concentration when started at 10 weeks of age (Fig 3). Neither blocker, however, changed RV collagen content (Fig 4) and concentration (Table 1) when administered from 15 to 20 weeks of age.

Enalapril and losartan when started at 10 or 15 weeks of age caused only small changes in LV elastin content (Figs 3 and 4) and concentration (Table 2). However, enalapril and losartan decreased both RV elastin content and concentration by about 50% and 35% when started at 10 or 15 weeks of age as compared with control rats (Table 2; Figs 3 and 4).

### Table 2. Changes in LV and RV Elastin Concentration During Chronic Volume Overload by Aortocaval Shunt and Effects of Enalapril and Losartan on These Changes

<table>
<thead>
<tr>
<th>Duration of Volume Overload</th>
<th>4 Weeks</th>
<th>10 Weeks</th>
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<tbody>
<tr>
<td></td>
<td>Prevention</td>
<td>Regression</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>1 Week</th>
<th>0-4 Weeks</th>
<th>5-10 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV elastin relative, % of LV weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.05±0.01</td>
<td>0.06±0.01</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>Control+enalapril</td>
<td>...</td>
<td>0.05±0.01</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>Control+losartan</td>
<td>...</td>
<td>0.04±0.01*</td>
<td>0.06±0.01*</td>
</tr>
<tr>
<td>Shunt</td>
<td>0.04±0.01</td>
<td>0.07±0.01</td>
<td>0.02±0.01§</td>
</tr>
<tr>
<td>Shunt+enalapril</td>
<td>...</td>
<td>0.06±0.01</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>Shunt+losartan</td>
<td>...</td>
<td>0.06±0.01†</td>
<td>0.06±0.01†</td>
</tr>
<tr>
<td>RV elastin relative, % of RV weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.16±0.03</td>
<td>0.19±0.02</td>
<td>0.12±0.02§</td>
</tr>
<tr>
<td>Control+enalapril</td>
<td>...</td>
<td>0.09±0.01*</td>
<td>0.09±0.01*</td>
</tr>
<tr>
<td>Control+losartan</td>
<td>...</td>
<td>0.10±0.01*</td>
<td>0.09±0.01*</td>
</tr>
<tr>
<td>Shunt</td>
<td>0.14±0.01</td>
<td>0.19±0.02</td>
<td>0.10±0.01</td>
</tr>
<tr>
<td>Shunt+enalapril</td>
<td>...</td>
<td>0.13±0.02†</td>
<td>0.12±0.01†</td>
</tr>
<tr>
<td>Shunt+losartan</td>
<td>...</td>
<td>0.08±0.01††</td>
<td>0.13±0.01††</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=5 to 7 per group). LV indicates left ventricular; RV, right ventricular.

*P<.05 vs control.
†P<.05 shunt+treatment vs shunt alone.
‡P<.05 shunt+treatment vs control+treatment.
§P<.05 control or shunt at 4 or 10 weeks vs control or shunt at 1 week.
||P<.05 control or shunt at 10 weeks vs control or shunt at 4 weeks.

![Fig 3](http://circ.ahajournals.org/)

**PREVENTION EXPERIMENT**

- **LV COLLAGEN**
  - Control: 1 mg
  - Control+enalapril: 2 mg
  - Control+losartan: 3 mg
- **LV ELASTIN**
  - Control: 0.5 mg
  - Control+enalapril: 1 mg
  - Control+losartan: 1.5 mg

**RV COLLAGEN**
- Control: 1.5 mg
- Control+enalapril: 2 mg
- Control+losartan: 2.5 mg

**RV ELASTIN**
- Control: 0.5 mg
- Control+enalapril: 1 mg
- Control+losartan: 1.5 mg

![Fig 4](http://circ.ahajournals.org/)

**REGRESSION EXPERIMENT**

- **LV COLLAGEN**
  - Control: 1 mg
  - Control+enalapril: 2 mg
  - Control+losartan: 3 mg
- **LV ELASTIN**
  - Control: 0.5 mg
  - Control+enalapril: 1 mg
  - Control+losartan: 1.5 mg

**RV COLLAGEN**
- Control: 1.5 mg
- Control+enalapril: 2 mg
- Control+losartan: 2.5 mg

**RV ELASTIN**
- Control: 0.5 mg
- Control+enalapril: 1 mg
- Control+losartan: 1.5 mg

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Effects of Cardiac Volume Overload by Aortocaval Shunt on LV and RV Collagen and Elastin

Aortocaval shunt for 1 week did not affect LV collagen content (Fig 2) or concentration (Table 1). Prolonged volume overload by aortocaval shunt for 4 and 10 weeks prevented the maturation-related increase in LV collagen accumulation as compared with control rats (Fig 2). Combined with the aortocaval shunt–induced LV hypertrophic growth, this resulted in a decrease in LV collagen concentration after 4 and 10 weeks as compared with control rats as well as with rats with a 1-week shunt (Table 1).

RV collagen content (Fig 2) and RV weight increased in parallel during the first 4 weeks of aortocaval shunt, and RV collagen concentration remained similar in shunt and control rats. Whereas in control rats RV collagen accumulation outpaced the RV growth from 4 to 10 weeks of age, resulting in a significant increase in RV concentration, no further RV collagen accumulation was found during chronic aortocaval shunt (Fig 2 and Table 1). As a result, after 10 weeks of an aortocaval shunt, RV collagen concentration was significantly lower compared with control rats of the same age (Table 1).

LV and RV elastin content increased in parallel with shunt-induced LV and RV growth up to 4 weeks of volume overload (Fig 2 and Table 2). Subsequently, LV and RV elastin accumulation in rats with chronic shunt (ie, 4 to 10 weeks after shunt surgery) decreased to levels of the control rats (Fig 2).

Effects of Enalapril and Losartan on Changes in LV and RV Collagen and Elastin Induced by Aortocaval Shunt

Prevention Experiment

Whereas in control rats both blockers decreased LV collagen accumulation, treatment for 4 weeks by both blockers in rats with aortocaval shunt did not change LV collagen content (Fig 3). However, since both blockers decreased LV weight, the LV collagen concentration increased as compared with untreated shunt rats to the level of untreated control rats (Table 1).

Treatment by either blocker for 4 weeks did not significantly affect increases in RV collagen content (Fig 3) and concentration (Table 1) induced by aortocaval shunt. Both blockers prevented the increases in LV and RV elastin content at 4 weeks after the shunt surgery (Fig 3).

Regression Experiment

Losartan but not enalapril, when started at 5 weeks after shunt, reversed to normal the decreases in LV collagen content (Fig 4) and concentration (Table 1) induced by chronic aortocaval shunt. Both blockers enhanced RV collagen accumulation during chronic aortocaval shunt (Fig 4) and prevented the decrease in RV collagen concentration observed after 10 weeks of shunt without treatment (Table 1).

Losartan but not enalapril blunted the decrease in LV elastin content (Fig 4) and concentration (Table 2) during chronic aortocaval shunt.

In contrast to the decrease in RV elastin content and concentration by the two blockers in control rats (Fig 4 and Table 2), neither blocker affected RV elastin content in shunt rats from 5 to 10 weeks after surgery (Fig 4). Since both blockers partially reversed RV hypertrophy in shunt rats,23 the RV elastin concentration actually increased compared with shunt and treated control rats (Table 2).

Effects of 10 Weeks of Volume Overload by Minoxidil Treatment on Cardiac Collagen and Elastin

RV collagen content increased in parallel with RV mass in rats on minoxidil for 10 weeks (Table 3), and RV collagen concentration did not differ from control rats (Table 3). In contrast, LV collagen content did not follow the increase in LV mass by minoxidil and actually decreased as compared with control rats, resulting in a decrease in LV collagen concentration (Table 3). Similarly, RV and LV elastin content and concentration decreased as compared with control rats (Table 3).

Volume Overload–Induced Changes in LV Pressures

Cardiac volume overload by an aortocaval shunt or by chronic minoxidil treatment increased LVEDP and decreased LVSP as compared with control rats (Table 4). Both enalapril and losartan started 3 days before or 5 weeks after the shunt/sham surgery and continued for 4 (prevention) and 5 (regression) weeks had minimal effect on LVEDP in control rats (Table 4). In shunt rats, enalapril and losartan similarly decreased LVEDP in both prevention and regression experiments. In rats on minoxidil, enalapril and losartan started at 5 weeks normalized LVEDP at 10 weeks (Table 4). In both control rats and rats with volume overload by an aortocaval shunt or chronic minoxidil treatment, the two blockers decreased LVSP, and the levels attained did not differ significantly between groups of rats (Table 4).

Discussion

The present study has the following major findings. (1) Cardiac volume overload by an aortocaval shunt or minoxidil decreased maturation-related collagen accu-

### Table 3. Effects of 10 Weeks of Minoxidil Treatment on LV and RV Collagen and Elastin Content and Concentration

<table>
<thead>
<tr>
<th></th>
<th>Total, mg</th>
<th>Concentration, % of Ventricular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LV collagen</td>
</tr>
<tr>
<td>Control</td>
<td>3.42±0.34</td>
<td>0.39±0.04</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>2.69±0.20*</td>
<td>0.22±0.03*</td>
</tr>
<tr>
<td>LV elastin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.29±0.01</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>0.28±0.03</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>RV collagen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.33±0.14</td>
<td>0.69±0.06</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>1.81±0.16*</td>
<td>0.66±0.06</td>
</tr>
<tr>
<td>RV elastin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.23±0.03</td>
<td>0.13±0.01</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>0.12±0.02*</td>
<td>0.05±0.01*</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=5 to 7 per group). LV indicates left ventricular; RV, right ventricular.

*P<.05 vs control.
mulation in the left ventricle but accelerated it in the right ventricle. (2) Both enalapril and losartan prevented the relative decreases in LV collagen content and concentration induced by shunt; losartan also reversed the decreased LV collagen induced by volume overload. (3) Both blockers had only minimal effect on the initial enhancement in RV collagen accumulation and potentiated the RV collagen accumulation during chronic aortocaval shunt. (4) Cardiac volume overload by aortocaval shunt for 4 weeks but not for 10 weeks accelerated LV and RV elastin accumulation. This initial increase in LV and RV elastin content was blocked by both blockers of the RAS.

**Effects of Enalapril and Losartan on Maturation-Related Changes in LV and RV Elastin and Collagen**

**Collagen**

During maturation from 10 to 20 weeks of age, increases in both LV and RV mass were accompanied by parallel increases in LV and RV collagen content up to 14 weeks of age (Table 1 and Fig 2). Whereas further LV growth was associated with a parallel increase in LV collagen content, RV collagen accumulation outpaced the RV growth from 14 to 20 weeks of age. As a result, RV and LV collagen concentration are similar from birth up to about 14 weeks of age, but RV collagen concentration becomes substantially higher as compared with LV collagen concentration in adult rats (present study; also Reference 30). Extending our findings on the effects of enalapril in rats from 5 to 10 weeks and from 10 to 15 weeks of age, the present study shows that both enalapril and losartan from 10 to 14 weeks as well as from 15 to 20 weeks of age decrease LV weight and LV collagen accumulation in parallel. Whereas blockade of the RAS caused parallel decreases in RV weight and collagen content from 5 to 10 weeks of age, such treatment had only minimal effects on the RV weight and collagen accumulation from 10 to 14 weeks and 15 to 20 weeks of age. Maturation-related growth and collagen accumulation of both the left ventricle and right ventricle may therefore involve the RAS up to 10 weeks of age. From 10 to 20 weeks of age, however, only LV but no longer RV growth and collagen accumulation appear to remain regulated by the RAS. Alternatively, since both blockers decreased LV peak systolic pressure by about 10 to 20 mm Hg over the treatment periods (Table 4), possibly did not affect the RV systolic pressure, the dissociation may point to systolic wall stress as the main determinant of further LV and RV growth and collagen accumulation over this period.

**Elastin**

Maturation-related changes in cardiac elastin have not yet been evaluated. From 10 to 20 weeks of age, the time course of LV and RV elastin accumulation appears to be similar to that in vessels, with ventricular elastin accumulation appearing to be completed by 10 to 14 weeks of age. Increases in LV and RV mass from 14 to 20 weeks of age were accompanied by no further increases in RV and LV elastin content, resulting in decreases in ventricular elastin concentration.

Enalapril and losartan started at 10 or 15 weeks of age similarly decreased RV elastin but had no significant effect on LV elastin. Thus, the effects of the two blockers of the RAS point to a clear dissociation in the regulation of LV versus RV elastin and collagen accumu-

**TABLE 4. Effects of Enalapril and Losartan on Volume Overload–Induced Changes in LVEDP and LVSP**

<table>
<thead>
<tr>
<th></th>
<th>Prevention 0-4 Weeks</th>
<th>Regression 5-10 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enalapril</td>
<td>Losartan</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1±0.4</td>
<td>2±1.3</td>
</tr>
<tr>
<td>Control+treatment</td>
<td>0±0.1</td>
<td>1±0.9</td>
</tr>
<tr>
<td>Shunt</td>
<td>7±0.5*</td>
<td>7±0.4*</td>
</tr>
<tr>
<td>Shunt+treatment</td>
<td>2±0.4†</td>
<td>4±0.8†</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>. .</td>
<td>. .</td>
</tr>
<tr>
<td>Minoxidil+treatment</td>
<td>. .</td>
<td>. .</td>
</tr>
<tr>
<td>LVSP, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>134±6</td>
<td>128±9</td>
</tr>
<tr>
<td>Control+treatment</td>
<td>128±3</td>
<td>112±8</td>
</tr>
<tr>
<td>Shunt</td>
<td>108±3*</td>
<td>112±3*</td>
</tr>
<tr>
<td>Shunt+treatment</td>
<td>110±4†</td>
<td>100±2*</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>. .</td>
<td>. .</td>
</tr>
<tr>
<td>Minoxidil+treatment</td>
<td>. .</td>
<td>. .</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=6 to 8). LVEDP indicates left ventricular end-diastolic pressure; LVSP, left ventricular peak systolic pressure.

*P<.05 vs control.
†P<.05 shunt or minoxidil+treatment vs shunt or minoxidil alone.
‡P<.05 shunt or minoxidil+treatment vs control+treatment.
mulation at this point in maturation: Blockade of the RAS inhibits collagen but not elastin accumulation in the left ventricle, and it inhibits elastin but not collagen accumulation in the right ventricle. Possible mechanisms for these dissociations cannot be addressed from our results.

**Effects of Volume Overload on LV and RV Collagen and Elastin**

**Collagen**

In contrast to the increases in LV collagen content and concentration caused by some models of pressure overload,4,12 LV collagen accumulation decreases in response to chronic cardiac volume overload induced by either aortocaval shunt (present study and Reference 14) or minoxidil (present study). In pressure overload, hypertrophic growth occurs in response to increased LV systolic wall stress.33 In contrast, in cardiac volume overload, the increase in LV diastolic wall stress33,34 determines LV hypertrophic growth, whereas LV systolic wall stress increases only to a minor extent.34 It is therefore possible that increases in systolic wall stress directly or indirectly potentiate collagen accumulation, but increases in diastolic wall stress have the opposite effects. The dissociation in the effects of cardiac volume overload on RV versus LV collagen accumulation is a new finding and supports the above hypothesis. Enhanced RV collagen accumulation during the initial period but no longer during chronic volume overload by aortocaval shunt may relate to the acute RV pressure overload (and thus probably increased RV systolic wall stress) shortly after opening of the aortocaval shunt and its decrease with chronic shunt.35,36 An aortocaval shunt increases RV systolic pressure to about 50 mm Hg.35 A similar increase in RV systolic pressure after pulmonary artery banding results in RV hypertrophy and increases collagen content by about 50% (similar to that observed after aortocaval shunt).37 The increase in RV diastolic pressure is clearly less as compared with LV diastolic pressure.35 The final outcome regarding collagen behavior in the left ventricle and right ventricle may depend on the balance between systolic and diastolic wall stresses. Changes in the balance between these two determinants (probably the result of changes in ventricular geometry and morphology and ventricular function) may explain observed differences in RV and LV collagen accumulation during different phases of aortocaval shunt.

In contrast to volume overload by aortocaval shunt, RV collagen content remained increased after 10 weeks of volume overload by minoxidil. This may relate to a more persistent increase in pulmonary artery and RV systolic pressure38 by minoxidil or a different time course of changes in collagen accumulation in this particular model.

**Elastin**

Cardiac volume overload by aortocaval shunt increased LV and RV elastin content as compared with control rats during the initial weeks. The subsequent decrease in LV and RV elastin content and concentration was similar in control and shunt rats. Since cardiac preload and PRA remained increased over the 10 weeks of volume overload,17,23 it appears that this maturation-related decrease in LV and RV elastin accumulation and concentration in both shunt and control rats is not determined by the RAS or hemodynamic changes.

**Role of the RAS in Volume Overload–Induced Changes in LV and RV Collagen and Elastin**

The RAS, aldosterone, and sympathetic nervous system may enhance collagen accumulation in pressure overload–induced cardiac hypertrophy.4,7,8,10-12,16,31 As we reported previously, PRA and cardiac renin (and probably angiotensin II) increase after the induction of an aortocaval shunt or start of minoxidil treatment and (may) remain increased during chronic volume overload by minoxidil and aortocaval shunt (depending on the size of aortocaval shunt).17,23,24 Both enalapril and losartan maintained LV collagen content and concentration in shunt rats at the level of control rats. Both blockers had only a small (NS) effect on the initial increase in RV collagen accumulation in shunt rats. These findings are consistent with the above-stated concept that increased diastolic wall stress inhibits and increased systolic wall stress potentiates collagen accumulation. Blunting of the increase in LV preload by the two blockers (Table 4) may explain the prevention of the decrease in LV collagen accumulation after shunt. The minimal effect of the two blockers on the increase in RV collagen in shunt rats may reflect their minimal effects on the RV systolic pressure.32

When treatment with enalapril or losartan was initiated in the chronic phase, both blockers similarly decreased LV systolic and diastolic pressures and reduced LV hypertrophy in parallel to their effect on LV preload.22 However, only losartan reversed the decrease in LV collagen accumulation during chronic volume overload. We are not aware of any nonspecific inhibitory/stimulatory effects of enalapril versus losartan on collagen that would explain the above results. In contrast, enalapril and losartan similarly potentiated RV collagen accumulation during chronic shunt. It is possible that the two blockers decreased RV diastolic wall stress (eg, by improving diastolic relaxation39) and thereby blunted its inhibitory effect on collagen accumulation. Both blockers prevented volume overload–induced increases in LV and RV elastin accumulation. These data, combined with the inhibitory effects of the blockers of the RAS on maturation-related increases in RV and LV elastin, suggest a role for the RAS in LV and RV elastin accumulation. Similar to its effect on LV collagen, only losartan prevented the decrease in LV elastin content during chronic volume overload. There is no obvious explanation for this difference between the two blockers.

**Conclusions**

In contrast to cardiac pressure overload, volume overload, even when accompanied by increased PRA, decreases LV collagen accumulation, suggesting an inhibitory effect of increased diastolic wall stress on collagen accumulation. Indeed, enalapril and losartan decrease LV preload and maintain LV collagen accumulation. In contrast to the decrease in LV collagen content and concentration, RV collagen accumulation was initially potentiated by volume overload, possibly due to acute RV pressure overload shortly after aortocaval shunt. Blockade of the RAS had minimal effect on the initial RV collagen accumulation and actually enhanced RV collagen during chronic shunt. In light of
these findings, the RAS appears to regulate volume overload–induced changes in LV and RV collagen accumulation by its hemodynamic effects and not by direct trophic effects.

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