Enalapril Prevents Cardiac Fibrosis and Arrhythmias in Hypertensive Rats

Marco Pahor, Roberto Bernabei, Antonio Sgadari, Giovanni Gambassi Jr., Pietro Lo Giudice, Licia Pacifici, Maria Teresa Ramacci, Costanza Lagrasta, Giorgio Olivetti, and Pierugo Carbonin

To evaluate the effects of hypertension on cardiac hypertrophy, on myocardial structure, and on ventricular arrhythmias, 27 3-month-old spontaneously hypertensive rats were treated with enalapril (10 mg/kg) daily for 11 months and compared with 26 untreated control rats. Systolic arterial pressure was significantly decreased in treated rats, and at the end of the experiment, it was 199±3 mm Hg (treated) versus 237±3 mm Hg (controls) (p<0.001). At this time, spontaneous arrhythmias and induced arrhythmias either by programmed electrical stimulation (train of stimuli +1 or 2 extrastimuli) or by trains of eight stimuli at decreasing coupling intervals were observed in isolated heart preparations. Comparing enalapril-treated and control rats, spontaneous arrhythmias (9 of 27 versus 20 of 26, respectively; p<0.01), programmed stimulation-induced arrhythmias (3 of 26 versus 12 of 23, respectively; p<0.01), and trains of stimuli-induced arrhythmias (4 of 26 versus 14 of 19, respectively, p<0.001) were less frequent in the enalapril group. Left ventricular weight was decreased in treated rats by 18% (p<0.001). Enalapril administration diminished the fraction of myocardium occupied by foci of replacement fibrosis normally occurring in control rats by 59% (p<0.001). Finally, a significant correlation was found between left ventricular weight, the extent of myocardial fibrosis, and the occurrence of ventricular fibrillation. It was concluded that chronic treatment with enalapril, which resulted in attenuation of systemic arterial pressure by limiting cardiac hypertrophy and myocardial fibrosis, decreases the propensity of the heart of hypertensive rats to arrhythmogenesis. (Hypertension 1991;18:148-157)

Spontaneously hypertensive rats (SHR) are more prone to develop ventricular arrhythmias (VA) and ventricular fibrillation (VF) than Wistar-Kyoto (WKY) rats or normal Wistar rats.1 Furthermore, coronary artery ligation in SHR increases significantly the duration of VF.2 The propensity of SHR to develop spontaneous or induced arrhythmias is greater in older animals and appears to be related to the duration of hypertension. In fact, an increased incidence of spontaneous, reperfusion-and programmed electrical stimulation (PES)-induced VA was observed in previous experiments in isolated hearts from 14-month-old SHR in comparison with hearts isolated from age-matched WKY rats or from 3-month-old SHR.3-5

Although the electrophysiological mechanism causing VA is still unclear, remodeling of the myocardium after myocyte cell loss appears to be of importance in the genesis of arrhythmias.6 In older SHR, cardiac pumping ability is compromised, myocardial fibrosis is markedly increased,7 and the spared myocytes are larger in size.8 The large amount of scarred tissue in the left ventricular myocardium of aged SHR may isolate small bundles of myocytes, leading to tissue anisotropy and eventually increasing the coupling resistance among myocytes, thus facilitating reentry circuits.6

This study was designed to test the hypothesis that myocardial damage and repair are responsible for the electrical instability in SHR hearts. Because left ventricular hypertrophy and left ventricular dysfunction can be prevented by long-term administration of angiotensin converting enzyme (ACE) inhibitors,9 enalapril was given to 3-month-old SHR, and the treatment was continued for 11 months. The results demonstrated that a moderate reduction in systolic arterial pressure was accompanied by a decrease of left ventricular hypertrophy, of myocardial scarring,
and incidence of VA. In addition, a significant correlation between VF and extent of myocardial fibrosis was demonstrated in both control and treated SHR. We conclude that the effects of enalapril are beneficial in decreasing the heart electrical instability by preserving myocardial structure in the SHR model.

Methods

Animals and Treatment

Male SHR at 3 months of age (Charles River, Calco, Como, Italy) were used. The animals were housed one per cage at a room temperature of 22±1°C and at 50–60% humidity with a 12-hour light/dark cycle schedule. Food (rat chow 4RF21, Mucedola SrL, Settimo Milanese, Milan, Italy) and tap water were freely available. Random codes were attributed to each animal.

One group of rats was given enalapril (Merck Sharp & Dohme, Rome, Italy) for 11 months (SHR-E). The drug was dissolved in drinking water, and the concentration was adjusted for the daily water intake and body weight to obtain an average daily dosage of 10 mg/kg. This dosage was chosen since preliminary experiments demonstrated that larger amounts of enalapril increased urea nitrogen and potassium content in the serum (unpublished data from our laboratory); also it was the lowest dose effective in reducing arterial pressure in 3-month-old SHR. A second group of animals receiving tap water for the entire experiment was used as control (SHR-C). To test the effect of short-term treatment with enalapril, one group of animals was given tap water for 10 months and 3 weeks and was subsequently treated for 1 week with 10 mg/kg enalapril in the drinking water (SHR-A). A further group of 14-month-old untreated SHR was used to test the effects of the electrical stimulation of the right ventricle on the induction of arrhythmias.

Systolic arterial pressure was measured every month at the same time of the day by the tail-cuff method using a Letica (models LE 5500, LE 5000A, and LE 5710, Hospitalet de Llobregat, Spain) apparatus in conjunction with a Letica polygraph (model LE 2000, Hospitalet de Llobregat).

After the last arterial pressure measurement, each animal was injected intraperitoneally with 5,000 IU heparin; after the rats were decapitated, the heart was rapidly excised and suspended for retrograde perfusion with a Langendorff apparatus.

Perfusion of Isolated Hearts

Each heart was perfused with a medium containing (in mM): NaCl 117.0, NaHCO₃ 23.0, KCl 4.6, Na₂HPO₄ 0.8, MgCl₂ 1.0, CaCl₂ 2.0, and glucose 5.5, equilibrated at 37°C and oxygenated with a 95% O₂–5% CO₂ gas mixture. The perfusion medium and the chamber containing the isolated heart were maintained at a constant temperature of 37°C. The hydrostatic aortic perfusion pressure was 10 kPa. The O₂ and CO₂ partial pressures and pH values were measured with a gas analyzer (model 213, Instrumentation Laboratories, Lexington, Mass.). Epicardial electrograms were recorded by an atrumatic unipolar electrode made with a cotton wick soaked in saline and impacted in a hollow cylinder (4 mm in diameter), placed on the anterior left ventricular wall 2 mm below the circumflex coronary artery and connected to an amplifier (model V 1205, E for M Instrument, Pleasantville, N.Y.). The reference electrode for the unipolar electrocardiographic (ECG) electrode was placed in the perfusion bath. The ECG signal was filtered for frequencies higher than 500 Hz and lower than 1 Hz. Left ventricular pressure was measured with a polyethylene catheter (0.5 mm in diameter) inserted in the ventricular cavity through the free left ventricular wall, with a pressure transducer (Statham P23, Gould, Inc., Oxnard, Calif.) connected to a pressure amplifier (model V 2203, E for M Instrument). All data were recorded on magnetic tape with a Panasonic Vetter model 420D recorder, Biomedical Mangoni, Pisa, Italy, and on paper with a simultrace recorder (model VR 12, E for M Instrument) for the analysis of arrhythmias. The coronary flow rate was measured by collecting the effluent.

Spontaneous Arrhythmias

After isolation, the hearts were observed for 20 minutes to allow stabilization of heart rate, left ventricular pressure, and coronary flow rate. During this period, spontaneously occurring VA were recorded. The diagnosis of VA was made according to the criteria described by the Lambeth Conventions. They were subdivided into three groups: 1) total VA including ventricular premature beats identifiable as premature QRS complexes in relation to the P wave (more than 5/min), ventricular tachycardia (VT) (at least five consecutive beats with large and aberrant QRS complexes and the absence of a preceding P wave), and VF (complete morphological irregularity in which individual QRS deflections cannot be distinguished from one another and for which a rate cannot be measured); 2) sustained VA with VT or VF lasting more than 30 seconds or irreversible; and 3) irreversible VA. The hearts with irreversible VF or VT were not used for the subsequent experiments.

Programmed Electrical Stimulation

The protocol used for PES has been already described elsewhere. Briefly, PES was effected using an electrical digital stimulator (model BM ST3, Biomedica Mangoni) connected to an atrumatic bipolar silver electrode placed on the anterior free wall of the left ventricle between the apex and the ECG recording electrode. To test the effects of right ventricular stimulation, a group of hearts from 14-month-old untreated SHR were stimulated by placing the electrode on the posterior aspect of the right ventricular wall midway from the right circumflex coronary artery and the apex. An extensible elastic wire gently pushed the electrode, ensuring a constant contact between the stimulating tips and the
epicardial surface (the force applied was approximately 200 mg). The heart was first stimulated with a train of eight or nine stimuli \( S_1 \). The threshold was measured in voltage units. Stimulation intensity was twice the threshold, stimulus length was 5 msec, and the \( S_1-S_2 \) interval was 150 msec. One or two extrastimuli \( (S_3, S_4) \) were then added at decreasing coupling intervals (steps of 10 msec between 150 and 70 msec and then steps of 5 msec) either until the refractory period was reached or until VT or VF developed. Each combination of stimuli was repeated twice.

According to duration and severity, PES-induced arrhythmias were arbitrarily classified as 1) VA including all cases of VT or VF; 2) sustained VA, when VA lasted more than 30 seconds or when VA were irreversible; and 3) only irreversible VA.

With regard to the type of arrhythmias, the cases were also classified as VT or VF. All hearts with VF only or with mixed forms of VT+VF were considered VF, whereas those with only VT without runs of VF were defined as VT.

### Trains of Stimuli

The hearts without irreversible VT or VF after PES were subjected to stimulation with trains of eight stimuli (TRAIN). The initial \( S_1-S_2 \) interval was 150 msec, and the stimulus length was 5 msec. Each train of stimuli was repeated twice, then the \( S_1-S_2 \) coupling interval was progressively decreased as during PES until either the heart was not driven by the train of stimuli or a VT or a VF developed. The classification of VA was the same as for PES-induced VA.

### Fixation Procedure

At completion of the electrophysiological test, all the SHR hearts were arrested in diastole with potassium chloride and fixed by perfusion of the coronary vasculature with a solution of glutaraldehyde (2.5%) and paraformaldehyde (2.0%) in phosphate buffer, 0.1 M, pH 7.4, for 3–6 minutes. The atria were excised, and the weights of the left ventricle, including the interventricular septum and the right ventricle, were recorded. Two apical slices, including both ventricles, were used for dry/wet weight determination.

### Tissue Sampling

Ten hearts in each group were randomly chosen and were examined for qualitative evaluation of myocardial damage. The ventricles were transversely cut into 10–15 slices perpendicular to the major axis of the heart. The two intermediate slices of each heart were radially cut to obtain 10–15 specimens extending from the endocardial to the epicardial surface of the free wall. The specimens were post-fixed in 1% osmium tetroxide dehydrated in acetone, and embedded in araldite.

### Morphometric Determination of Myocardial Fibrosis

For each rat, sections (1 \( \mu \)m thick) were cut from each of the 10 embedded tissue blocks of the left ventricle containing the entire thickness of the wall. The sections were then stained with methylene blue and safranin for quantitative measurements. Ten adjacent fields from the endocardium, mesocardium, and epicardium were examined in each rat at a calibrated magnification of \( \times 250 \) with a reticle containing 42 sampling points. This reticle defined an uncompressed tissue area of 148.000 \( \mu \)m\(^2\) that was used to determine the volume fraction of perivascular and replacement fibrosis by point counting. A minimum of 120 fields per heart was counted.

### Table 1. Body Weight and Heart Weight Measurements

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SHR-C ((n=26))</th>
<th>SHR-E ((n=27))</th>
<th>SHR-A ((n=12))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>419±7</td>
<td>412±7</td>
<td>408±5</td>
</tr>
<tr>
<td>Heart weight (mg)</td>
<td>1,568±32</td>
<td>1,328±37*</td>
<td>1,556±47</td>
</tr>
<tr>
<td>Left ventricular weight (mg)</td>
<td>1,274±24</td>
<td>1,043±29*</td>
<td>1,302±38</td>
</tr>
<tr>
<td>Right ventricular weight (mg)</td>
<td>294±13</td>
<td>285±13</td>
<td>254±16</td>
</tr>
<tr>
<td>Heart weight/body weight (mg/g)</td>
<td>3.7±0.1</td>
<td>3.2±0.1*</td>
<td>3.8±0.1</td>
</tr>
</tbody>
</table>

SHR-C, control spontaneously hypertensive rats; SHR-E, spontaneously hypertensive rats treated with enalapril for 11 months; SHR-A, spontaneously hypertensive rats treated with enalapril for 1 week.

*\(p<0.05\) vs. control.
Statistical Analysis

Results are expressed as mean±SEM. One-way analysis of variance was used to determine the significance of differences between the means. Scheffe's correction for multiple comparisons was applied when more than two groups were compared. Distributions of discrete variables were compared by means of the x^2 test with Yates' correction when the count in a single cell of the contingency table was less than five. Analysis of variance for repeated measures was used to test the effect of treatment on arterial pressure over time. Discriminant analysis was used to test the correlations between different types of fibrosis, systolic arterial pressure, and the occurrence of VF. A value of p<0.05 was considered statistically significant. All statistical procedures were performed with SPSS/PC.

Results

Baseline Characteristics

Figure 1 shows the changes in systolic arterial pressure detected in SHR-C and in SHR-E throughout the experiment. At the beginning of treatment, systolic arterial pressure was approximately 220 mm Hg in both groups of rats. At the end of the first month of treatment, a significant decrease in pressure values (SHR-C, 224±5 mm Hg; SHR-E, 210±4 mm Hg; -6%, p<0.02) was already seen in SHR-E and a significant difference persisted for the following 10 months. At the end of the experiment, systolic arterial pressure reached a value of 237±3 mm Hg in SHR-C and averaged 199±3 mm Hg in SHR-E (-16%, p<0.001). Also in SHR-A, after 1 week of treatment with enalapril, the systolic pressure decreased from 230±7 mm Hg to 184±5 mm Hg (-20%, p<0.001).

Body weight was not affected by the drug, but a significant decrease in heart weight (-15%), left ventricular weight (-18%), and heart weight/body weight ratio (-14%) was seen in SHR-E, whereas those parameters were not significantly modified in the SHR-A (Table 1). No changes were found in the weight characteristics of the right ventricle (Table 1). Dry weight to wet weight determinations demonstrated that the changes in weight were due to changes in proteins with no accumulation of water within the myocardial tissue (SHR-C, 0.22±0.04; SHR-E, 0.21±0.04; NS).

In isolated heart preparations, heart rate, left ventricular pulse pressure, and total coronary flow rate were similar in the three groups of rats (Table 2). In contrast, coronary flow rate per gram of ventricular weight was significantly higher in the SHR-E (+13%, p<0.01).

Ventricular Arrhythmias

Spontaneous ventricular arrhythmias. Table 3 summarizes the frequency of episodes of ventricular arrhythmias detected in the isolated heart preparations in control and treated rats. In SHR-C, 77% of the hearts had spontaneous VA during the stabilization period in comparison with only 33% of the hearts from SHR-E (p<0.01). An example of spontaneous VT is shown in Figure 2. SustainedVF was detected in four of 26 hearts of SHR-C and in one of 27 hearts of SHR-E. This difference, however, was not statistically significant. Three hearts of the control group and one of the treated group had an irreversible VF. No significant difference in the incidence of spontaneous VA was observed between SHR-C and SHR-A.

Programmed electrical stimulation–induced ventricular arrhythmias. No VA were induced by the stimuli train alone. All cases with PES-induced VA exhibited VF (this includes also mixed formsVF+VT as in the example shown in Figure 3). In SHR-C, 52% of the hearts and only 12% of the hearts of SHR-E had PES-induced VA (p<0.01) (Table 3). No sustained VF or irreversible VA were observed in hearts from SHR-E. On the contrary, 1 week of treatment with enalapril did not affect significantly the incidence of PES-induced VA (Table 3). In all the experiments in which VA were not irreversible, PES-induced VA were reproducible by the same combination of stimuli in 67% of hearts. When the hearts were stimulated from the right ventricle, the incidence of PES-

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SHR-C (n=26)</th>
<th>SHR-E (n=27)</th>
<th>SHR-A (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate</td>
<td>266±6</td>
<td>271±6</td>
<td>262±10</td>
</tr>
<tr>
<td>Left ventricular pulse pressure (kPa)</td>
<td>9.20±0.21</td>
<td>9.45±0.29</td>
<td>9.90±0.24</td>
</tr>
<tr>
<td>Coronary flow rate (ml/min)</td>
<td>13.9±0.4</td>
<td>13.4±0.4</td>
<td>14.0±0.7</td>
</tr>
<tr>
<td>Coronary flow rate/heart weight (ml/g.min)</td>
<td>8.9±0.3</td>
<td>10.1±0.3*</td>
<td>9.2±0.6</td>
</tr>
</tbody>
</table>

SHR-C, control spontaneously hypertensive rats; SHR-E, spontaneously hypertensive rats treated with enalapril for 11 months; SHR-A, spontaneously hypertensive rats treated with enalapril for 1 week. *p<0.05 vs. control.

![Figure 2. Representative electrocardiographic recording of spontaneously hypertensive rat from the control group shows an example of spontaneously occurring arrhythmias (upper panel). Sinus rhythm (P indicates the P waves) is followed by brief runs of reversible ventricular tachycardia returning to normal sinus rhythm (lower panel).](image-url)
induced VA was similar to that of SHR-C and significantly higher than that of SHR-E (Table 3).

**TRAIN-induced ventricular arrhythmias.** The 19 SHR-C hearts and the 26 SHR-E hearts that did not have irreversible VA after PES were subjected to TRAIN. The incidence of VA (Table 3) was 74% in the control group and 15% in the treated group (p<0.001). VF was observed in 58% and in 12% (p<0.01) of the two groups of hearts, respectively. The remaining cases of VA were represented by VT alone (Figure 4). No sustained or irreversible VA were observed in SHR-E. In the 13 cases with reversible VA, these were reproducible by the same stimulation rate as in 69% of the hearts. One week of treatment with enalapril did not modify significantly the incidence of TRAIN-induced VA (Table 3). No significant difference in TRAIN-induced VA was observed between right and left ventricular stimulation (Table 3).

**TABLE 3. Frequency of Ventricular Arrhythmias in Isolated Spontaneously Hypertensive Rats Heart Preparations**

<table>
<thead>
<tr>
<th>Type of VA</th>
<th>SHR-C</th>
<th>SHR-E</th>
<th>SHR-A</th>
<th>SHR-R</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spontaneous VA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20/26 (77%)</td>
<td>9/27 (33%)*</td>
<td>7/12 (58%)</td>
<td>9/14 (64%)</td>
</tr>
<tr>
<td>Sustained</td>
<td>4/26 (15%)</td>
<td>1/27 (4%)</td>
<td>1/12 (8%)</td>
<td>2/14 (14%)</td>
</tr>
<tr>
<td>Irreversible</td>
<td>3/26 (12%)</td>
<td>1/27 (4%)</td>
<td>1/12 (8%)</td>
<td>2/14 (14%)</td>
</tr>
<tr>
<td><strong>PES-induced VA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>12/23 (52%)</td>
<td>3/26 (12%)*</td>
<td>6/11 (55%)</td>
<td>8/12 (67%)†</td>
</tr>
<tr>
<td>Sustained</td>
<td>5/23 (22%)</td>
<td>0/26 (0%)‡</td>
<td>2/11 (18%)</td>
<td>2/12 (17%)</td>
</tr>
<tr>
<td>Irreversible</td>
<td>3/23 (13%)</td>
<td>0/26 (0%)‡</td>
<td>1/11 (9%)</td>
<td>2/12 (17%)</td>
</tr>
<tr>
<td><strong>TRAIN-induced VA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14/19 (74%)</td>
<td>4/26 (15%)§</td>
<td>5/10 (50%)</td>
<td>8/10 (80%)‖</td>
</tr>
<tr>
<td>Sustained</td>
<td>5/19 (26%)</td>
<td>0/26 (0%)‡</td>
<td>1/10 (10%)</td>
<td>2/10 (20%)</td>
</tr>
<tr>
<td>Irreversible</td>
<td>5/19 (26%)</td>
<td>0/26 (0%)‡</td>
<td>1/10 (10%)</td>
<td>2/10 (20%)</td>
</tr>
</tbody>
</table>

Values are number of hearts with ventricular arrhythmias/all hearts examined (%). SHR-C, control spontaneously hypertensive rats; SHR-E, spontaneously hypertensive rats treated with enalapril for 11 months; SHR-A, spontaneously hypertensive rats treated with enalapril for 1 week; SHR-R, untreated 14-month-old spontaneously hypertensive rats stimulated from the right ventricle; VA, ventricular arrhythmias; PES, programmed electrical stimulation; TRAIN, trains of stimuli.

*p<0.05, †p<0.01, ‡p<0.001 vs. SHR-C.

Shr-C, untreated 14-month-old spontaneously hypertensive rats stimulated from the right ventricle; VA, ventricular arrhythmias; PES, programmed electrical stimulation; TRAIN, trains of stimuli.

The hearts that experienced VF in at least one experimental condition (baseline perfusion, PES, or TRAIN) were 20 of 26 (77%) in the SHR-C group and five of 27 (19%) in the SHR-E group (p<0.001).

**Morphology**

Light microscopic examination of the left ventricular myocardium of both groups of rats revealed the presence of discrete areas of replacement fibrosis scattered throughout the wall (Figure 5A). Perivascular accumulation of connective tissue around the intramural vessels (Figure 5A) was an additional marked alteration of the myocardium. To measure the extent of replacement and perivascular fibrosis, both lesions were morphometrically evaluated and the results illustrated in Figure 6. The amount of perivascular fibrosis in the entire wall was smaller in the SHR-E animals (−25%), although this result was not significantly different between the two groups of SHR-C and SHR-E.

**FIGURE 3.** Representative electrocardiographic recording of spontaneously hypertensive rat from the control group shows an example of arrhythmias induced with programmed electrical stimulation (upper panel) and its magnified segments (a, 0.45 second; b, 1 second; and c, 0.75 second; lower panel) during normal sinus rhythm. Panel a: Electrocardiographic deflections (P) due to atrial activation can be clearly seen before each ventricular complex. Panel b: A train of stimuli $S_1$, followed by one extrastimulus $S_2$ induces ventricular tachycardia. Panel c: Ventricular fibrillation, finally spontaneously returning to sinus rhythm. $S_1-S_2$ interval, 150 msec; $S_1-S_1$ interval, 55 msec.
animals (Figure 6). By contrast, a significant reduction in the volume percent of replacement fibrosis was found in the endocardium (−58%) and in the mesocardium (−75%) of SHR-E in comparison with SHR-C (Figure 6). In the epicardial layer, the fraction of myocardium occupied by fibrotic tissue was also smaller (−28%) in SHR-E, although this value was not significantly different from that found in SHR-C. In the entire ventricular wall, the treatment with enalapril was able to reduce the amount of fibrotic tissue by 50% (Figure 7).

**Ventricular Fibrillation, Arterial Pressure, Morphology, and Left Ventricular Weight**

For the 20 rats in which the amount of myocardial fibrosis was evaluated, discriminant analysis was used to assess whether VF in at least one experimental condition (control perfusion, PES, or TRAIN) was related to systolic arterial pressure and severity of myocardial fibrosis (Table 4). Of the 20 randomly chosen hearts, one treated and nine control hearts had VF. Either left ventricular replacement fibrosis or systolic arterial pressure was correlated with the episodes of VF. The amount of replacement fibrosis in the mesocardium was the strongest predictor of VF, followed by systolic arterial pressure and by endocardial and epicardial replacement fibrosis. Conversely, perivascular fibrosis was not related to VF. Furthermore, considering all the experiments (N=53), the hearts that exhibited VF had significantly heavier left ventricles than hearts with no episodes of VF (Figure 8).

**Discussion**

The results of the present study demonstrated that 11 months of enalapril treatment in SHR leads to a final reduction of nearly 40 mm Hg in systolic arterial pressure and prevents left ventricular hypertrophy and structural alterations. The preservation of myocardial integrity was accompanied by a drastic reduction in frequency, duration, and severity of spontaneous or stimulated arrhythmias in the isolated heart preparation. In the present study systolic arterial pressure remained between 190 and 210 mm Hg for 10 months in SHR-E, with an average final value of 199 mm Hg. Nevertheless, myocardial structure was significantly preserved, heart and left ventricular weight were decreased almost proportionally to the reduction of arterial pressure, and the electrical instability was considerably decreased. Thus, normalization of arterial pressure does not appear to be necessary to preserve myocardial structure and to reduce the propensity of the heart to produce arrhythmias.

The positive effect of enalapril treatment may be attributed to the reduction of the mechanical load on the myocardium. In the SHR model, a poor, albeit significant, correlation between systemic arterial pressure and ventricular mass has been found, and a reduction in systemic pressure was detected with increased ventricular mass. However, in the older animals of this strain, significant myocardial damage has always been found with a different degree of hypertrophy of the remaining myocytes in the presence of depressed global cardiac function. These results are consistent with a deleterious effect of the hypertensive state on myocardial structure, resulting in hypertrophy of myocytes and increased connective tissue accumulation. Although the relations between these two phenomena are still unclear, by unloading the left ventricular myocardium, myocardial oxygen demand should decrease, and the intramural physical stress on a per cell basis should be markedly reduced, avoiding the potential damage of physical stresses.

The modest (+13%), albeit significantly higher, coronary flow rate per myocardial mass unit found in the group of treated SHR (Table 2) is consistent with the results obtained by other investigators, who found that 5 months' treatment of 3-month-old SHR with the ACE-inhibitor cilazapril improved the maximal coronary flow rate in isolated perfused hearts and increased the capillary density in the myocardium.

However, a direct action of enalapril on the myocardium cannot be excluded. In fact, ACE inhibitors can counteract the multiple effects of angiotensin II on the myocardium. Recently, Linz and coworkers demonstrated that a nonantihypertensive low dose of the ACE-inhibitor ramipril determines the same complete regression of cardiac hypertrophy as seen in a group receiving the antihypertensive dose of ACE inhibitor. In hypertensive rats, Chevillard et al. observed that 10 mg/kg of enalapril (i.e., the same drug dose we used) reduced but did not normalize the arterial pressure and simultaneously caused a marked inhibition of myocardial converting enzyme activity.

**Antiarrhythmic Action of Enalapril**

Malignant VA seems to be the result of the interplay of three main factors: the structural alterations of the myocardium that represent substrate factors, spontaneous or PES-induced ventricular depolarizations that are indicated as trigger factors, and
finally, sympathetic activation that can be considered as a modulating factor.

In the isolated rat heart, we have previously demonstrated that PES-induced VA are more frequent and severe in the presence of myocardial abnormalities such as regional ischemia, hypoxia, or cardiac hypertrophy due to long lasting hypertension. It has been shown that 3- and 14-month-old normotensive Wistar-Kyoto rats in the same experimental setup have no spontaneous VA and that PES-induced VA are significantly increased only in 14-month-old SHR compared with 3-month-old SHR or with age-matched normotensive controls. Thus, it was concluded that the duration of hypertension was the main factor influencing the occurrence of ventricular arrhythmias in this experimental model.
Although we cannot completely exclude that local autonomic activation has played a role in the genesis of VA since catecholamines may still be released from nerve terminals located in the myocardium after stimulation,\textsuperscript{22} it is unlikely that the sympathetic factor was a major determinant of VA. In fact, in previous experiments in isolated hearts from SHR and from normotensive rats we found no correlation between myocardial catecholamine content and induction of VA.\textsuperscript{3}

In the present study a significant correlation between the extent of myocardial fibrosis and the incidence of VF in both groups of SHR has been found. Myocardial structure in old SHR is modified by the presence of large areas of replacement fibrosis located in the mesomyocardium and endomyocardium, similar by qualitative appearance to fibrotic areas that can be seen in the surviving myocardium after one infarction. In the chronic stage of myocardial infarction, the anatomic reentry circuit has been proved to be the substrate factor for spontaneous and PES-induced VA.\textsuperscript{23,24} Although myocyte cell loss after one infarction is repaired by large areas of fibrotic tissue, the surviving myocardium is composed of many areas of heterogeneous tissue in which cardiac fibers are embedded within connective tissue.\textsuperscript{24} Because similar structural alterations were found in the myocardium of old SHR, the same arrhythmogenic potential for reentry can be operative in SHR left ventricles. Furthermore, in the present experiments it has been demonstrated that VA could be equally induced by right or left ventricular stimulation. Thus, the induced VA represent a really increased propensity to arrhythmogenesis of the older SHR hearts and are not an artifactual result of an interaction between the fibrotic tissue of the left ventricle and the pacing electrode.

Enalapril has no direct antiarrhythmic action on the isolated heart.\textsuperscript{25} In addition, we have observed that 1 week of treatment of 14-month-old SHR with enalapril did not modify myocardial hypertrophy (Table 1) or the incidence of PES- and TRAIN-induced VA (Table 3). Similar negative results have been recently described in a model of sudden death in hypertensive dogs with left ventricular hypertrophy after 5 days of treatment with enalapril.\textsuperscript{26} Instead, in our model with a long-term treatment, myocardial fibrosis, hypertrophy, and incidence of VA were significantly reduced in hearts from SHR-E. In other words, enalapril, by preventing the development of myocardial damage (i.e., the anatomic arrhythmogenic substrate), might have diminished the propensity to reentry and thus, the incidence of spontaneous and induced VA.

It should be pointed out, however, that cardiac hypertrophy is not uniform, and the magnitude and duration of hypertensive state are important determinants of the functional and structural changes occurring in the myocardium. Recent studies in hypertensive dogs have shown that superimposed regional ischemia may produce a pronounced conduction abnormality and facilitate VT and sudden death.\textsuperscript{26,27} In this model of renal hypertension of short duration, hypertrophy in the absence of myocardial damage is likely to be the determinant of greater conduction delay in the ischemic zone. On the other hand, in infarcted human hearts,\textsuperscript{24} it has been demonstrated that arrhythmogenic tissue is generally near the infarct and is composed of both

![Figure 6](https://hyper.ahajournals.org/)

**Table 4.** Correlation Between Occurrence of Ventricular Fibrillation, Systolic Arterial Pressure and Amount of Different Types of Fibrosis in all Rats Independently From Treatment.

<table>
<thead>
<tr>
<th>Type of fibrosis</th>
<th>NO VF (n = 10)</th>
<th>VF (n = 10)</th>
<th>Correlation*</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesocardial replacement</td>
<td>2.2±0.5</td>
<td>9.6±0.9</td>
<td>0.77</td>
<td>0.0000</td>
</tr>
<tr>
<td>Systolic pressure†</td>
<td>198±6</td>
<td>233±6</td>
<td>0.52</td>
<td>0.0002</td>
</tr>
<tr>
<td>Endocardial replacement</td>
<td>4.9±1.2</td>
<td>15.7±2.2</td>
<td>0.48</td>
<td>0.0004</td>
</tr>
<tr>
<td>Epicardial replacement</td>
<td>4.5±0.8</td>
<td>6.1±0.5</td>
<td>0.30</td>
<td>0.0144</td>
</tr>
<tr>
<td>Mesocardial perivascular</td>
<td>2.3±0.3</td>
<td>4.2±0.9</td>
<td>0.22</td>
<td>0.0617</td>
</tr>
<tr>
<td>Epicardial perivascular</td>
<td>1.5±0.3</td>
<td>2.0±0.4</td>
<td>0.11</td>
<td>0.3305</td>
</tr>
<tr>
<td>Endocardial perivascular</td>
<td>2.9±0.6</td>
<td>4.2±1.3</td>
<td>0.10</td>
<td>0.3941</td>
</tr>
</tbody>
</table>

Variables are sorted by decreasing correlation coefficients.

*Discriminant analysis was used to compute correlations. Numbers indicate pooled-within-group correlations between discriminating variables and occurrence of ventricular fibrillation (VF).
†Probability values indicate significance of univariate statistic.
$Systolic arterial pressure (mm Hg)$ as measured after 11 months of treatment.
normal myocardial cells and fibrotic tissue. Furthermore, it has been shown that patients with left ventricular hypertrophy and VA have more subendocardial fibrosis than patients without arrhythmias.\(^28\) It cannot be excluded, however, that the antiarrhythmic effect of enalapril is also mediated by other factors. By enhancing the amplitude of oscillatory afterdepolarizations, programmed electrical stimulation can facilitate the occurrence of VA due to both triggered activity and reentry.\(^29\) Furthermore, it has been observed that myocardial cells of hypertrophic hearts from renal hypertensive rats are more prone to develop afterdepolarizations and triggered activity.\(^30\) Accordingly, in our experiments, it might be hypothesized that enalapril has also prevented VA by decreasing the propensity of the hypertrophied myocardium to afterdepolarizations and triggered activity. Thus, although different mechanisms of arrhythmogenesis and its prevention can be operative in different models of cardiac hypertrophy, the results of the present experiments strongly suggest that myocardial hypertrophy and myocardial damage have to be considered important substrates in the genesis of ventricular arrhythmias.

**Clinical Implications**

Although caution should be taken in extrapolating to humans results obtained with experimental animals, our previous observations\(^3\) and the present results in the isolated heart are in agreement with clinical observations in hypertensive patients where programmed stimulation induced more ventricular arrhythmias in those with ventricular hypertrophy than in those without hypertrophy.\(^31\) The present data are likely to support three different suggestions pertinent to human cardiac pathology.

First, left ventricular hypertrophy is an independent risk factor for cardiovascular morbidity and mortality,\(^32\) and a clear association of left ventricular hypertrophy with an excess incidence of complex VA and sudden death has been demonstrated.\(^34\)\(^,\)\(^35\) Our results showed that the propensity to severe arrhythmias (VF) is associated with left ventricular hypertrophy and fibrosis. Thus, long-term antihypertensive therapy, by decreasing left ventricular hypertrophy and myocardial fibrosis, could reduce the risk of sudden death. This hypothesis has been recently suggested by McLenachan and Dargie.\(^36\)

Second, it has been suggested that an excessive blood pressure reduction might be deleterious to the patient.\(^37\)\(^,\)\(^38\) The fact that enalapril had beneficial effects on the myocardial morphology and on VA with only a limited decrease of systolic arterial pressure might be of clinical relevance.

Finally, the antidisrhythmic effect of ACE inhibitors, which is likely to be independent of any established direct electrophysiological action,\(^39\) may be of greater therapeutic benefit in preventing complex VA and possibly sudden death in patients with cardiac hypertrophy than traditional antiarrhythmic drugs, which can potentially have proarrhythmic effects.\(^40\)\(^,\)\(^41\)

**Acknowledgments**

We thank Giovanni Vagni and Gianni Quattrini for their skillful technical assistance.

**References**


Key Words: enalapril • arrhythmias • hypertension • fibrosis • spontaneously hypertensive rats.
Enalapril prevents cardiac fibrosis and arrhythmias in hypertensive rats.
M Pahor, R Bernabei, A Sgadari, G Gambassi, Jr, P Lo Giudice, L Pacifici, M T Ramacci, C Lagrasta, G Olivetti and P Carbonin

doi: 10.1161/01.HYP.18.2.148

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/18/2/148