Inhibition of Angiotensin-Converting Enzyme Reduces Susceptibility of Hypertrophied Rat Myocardium to Ventricular Fibrillation

Yasuyuki Shimada, MD*; Suba Gunasegaram, BSc; Hiroyuki Yokoyama, MD; Metin Avkiran, PhD

Left ventricular (LV) hypertrophy increases susceptibility to reperfusion arrhythmias and the angiotensin-converting enzyme inhibitor, ramipril, may reduce that susceptibility via regression of LV hypertrophy. Rats (n=12 per group) were subjected to abdominal aortic constriction (AC) or sham-operation (SH) and from 3 to 6 weeks after surgery, 3 AC groups received ramipril (0.01, 0.1, or 1 mg/kg per day) while the SH and 1 AC group received vehicle. Six weeks after surgery (ie after 3 weeks of treatment), the hearts were excised and subjected to independent Langendorf perfusion of left and right coronary beds. The left coronary bed was then subjected to ischemia (7 min) and reperfusion (5 min). Hypertrophied hearts from the vehicle AC group showed a significant increase in the incidence of reperfusion-induced ventricular fibrillation (VF) compared with control hearts from the SH group (92%* vs 33%; *p<0.05); this difference was abolished by ramipril (42%, 50%, and 42%, at 0.01, 0.1, or 1 mg/kg per day, respectively). The LV weight/body weight ratio was significantly increased in all AC groups (regardless of ramipril treatment) relative to the SH group. At the cellular level, myocyte length was significantly increased in the vehicle AC group, but was normalized by ramipril treatment (1 mg/kg per day). At the molecular level, atrial natriuretic factor (ANF) mRNA expression was also significantly increased in the vehicle AC group, but was again normalized by ramipril treatment (1 mg/kg per day). In conclusion, short-term treatment with ramipril reduced susceptibility to severe ventricular arrhythmias in hypertrophied rat hearts. This protection was achieved in the absence of a significant reduction in LV weight, but was accompanied by regression of myocyte hypertrophy, as reflected by reductions in cell size and ANF expression. (Circ J 2002; 66: 1045–1053)

Key Words: Angiotensin-converting enzyme inhibitor; Atrial natriuretic factor; Hypertrophy; Ischemia induced arrhythmias

An increasing number of clinical studies have revealed that cardiac hypertrophy is a powerful risk factor for sudden cardiac death, probably because of the greater vulnerability to severe ventricular arrhythmias associated with myocardial ischemia.1–5 Experimental studies have shown that left ventricular (LV) hypertrophy increases vulnerability to ventricular arrhythmias during both ischemia6–9 and reperfusion10,11 in different animal species. Although hypertrophy is defined as an increase in cell size, ventricular hypertrophy is achieved by increases in both myocyte size and the volume of nonmyocyte components such as interstitial collagen.12,13 Indeed, studies with various animal hypertrophy models have revealed that not only intrinsic changes in myocytes (see review by Pye14) but also myocardial fibrosis15–17 significantly contribute to the increased vulnerability to ventricular arrhythmias. Recently, treatments for hypertension, including drugs such as angiotensin-converting enzyme inhibitors (ACEI) and angiotensin II AT1 antagonists18 have been shown to suppress susceptibility to ventricular arrhythmias through regression of LV hypertrophy.15–17,19 However, the relative contribution of myocyte and nonmyocyte components to arrhythmogenesis in hypertrophied myocardium has never been examined, and how regression of hypertrophy mediates attenuation of arrhythmias is still unclear.

The present study was designed to determine whether (1) treatment with an ACEI, ramipril, reduces arrhythmia vulnerability in the rat heart with established pressure overload-induced LV hypertrophy, and (2) any anti-arrhythmic effect of ramipril is accompanied by changes in the myocyte and/or non-myocyte fractions of the hypertrophied LV myocardium. To achieve these objectives, we used a rat model in which LV pressure overload is induced by abdominal aortic constriction with a metal clip20 and a dual coronary perfusion model that enables induction of regional ischemia in the left coronary bed supplying the hypertrophied myocardium.21 Hydroxyproline content, as an index of interstitial fibrous tissue volume,22 and the size of isolated myocytes were measured in both left and right ventricles. To monitor the development and regression of myocardial hypertrophy at the molecular level, we measured the LV expression of atrial natriuretic factor (ANF) mRNA.23,24 Our results indicated that short-term treatment with ramipril reduced susceptibility to reperfusion-induced ventricular fibrillation (VF) in hearts with established LV hypertrophy. Furthermore, this protection was achieved without a reduction in LV mass (but an
increase in hydroxyproline content) and was accompanied by the regression of myocyte hypertrophy, as reflected by cellular and molecular indices.

**Methods**

This investigation was performed in accordance with the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986, published by Her Majesty's Stationery Office, London.

**Induction of Aortic Constriction**

Male Wistar rats with a similar mean starting body weight were used in each study subsection (Table 1). Animals were anesthetized with fentanyl (0.3 ml/kg body weight im) and diazepam (2.5 mg/kg body weight ip). Via a small abdominal incision, the aorta was isolated and cleared of surrounding tissue. A small titanium clip (Atrauclip, Rusch UK Ltd, High Wycombe, UK) was placed around the suprarenal aorta with the aid of a specially adapted pliers set to a width of 0.45 mm. The incision was closed with individual vicryl sutures and the rats left to recover under a heating lamp. The rats were closely observed for 6 h post operation to ensure complete recovery from the anesthetic. Sham-operated rats were randomly assigned and underwent the same procedure, except for the placing of the titanium clip. Following recovery from anesthesia, the rats were housed 4–6 to a cage with free access to food and water. They were maintained for the duration of the study in a room kept at 25°C with a 12 h light–dark cycle.

**Drug Treatment Protocol**

From 3 to 6 weeks after surgery, sham-operated animals (SH) received vehicle (drinking water), and animals with aortic constriction (AC) received either vehicle or 0.01, 0.1, or 1 mg/kg per day po of Ramipril (AC+R0.01, AC+R0.1, and AC+R1, respectively) Starting at 3 weeks after surgery.

**Reperfusion-Induced Arrhythmias**

**Dual Coronary Perfusion of Isolated Rat Hearts**

After BP was measured, the hearts were excised, and the left and right coronary arteries were subjected to independent perfusion as described in detail by Avkiran and Curtis. In brief, heparin (200 IU) was injected into the right femoral artery through a cannula, which was inserted into a fluid-filled catheter. They were recorded on an ink-jet recorder (model RS3400, Gould Electronic Ltd, Ilford, UK) after BP was stable for 10 min.

Throughout the experiment, each coronary bed was perfused at a constant pressure (equivalent to 75 mmHg) with oxygenated perfusion solution drawn from a temperature-regulated reservoir (37°C). The perfusion solution was of the following composition (in mmol/L): NaCl, 118.5; NaHCO3, 25.0; KCl, 3.2; MgSO4, 1.2; KH2PO4, 1.2; CaCl2, 1.4; and glucose, 11.0. The solution was filtered (pore size, 5μm) before use and gassed continuously with 95% O2/5% CO2 (pH 7.4 at 37°C). Flow to each coronary bed was monitored by in-line flow detectors (Transonic T206 Animal Research Flowmeter with 1 N probes, Transonic Systems Inc, NY, USA) with a linear detection range of 0.05–30 ml/min. The hearts were housed in a temperature-regulated chamber at 37°C and were continuously superfused at 8 ml/min.

**Perfusion Protocol**

After a 15-min aerobic perfusion, the hearts were subjected to 7 min of regional ischemia by flow stoppage to the left coronary bed; the left coronary bed was then reperfused for 5 min, during which arrhythmias were assessed.

**Diagnosis of Reperfusion-Induced Arrhythmias**

Arrhythmias were diagnosed via a unipolar electrocardiogram (ECG) that was obtained through a silver electrode inserted into the free wall of the left ventricle and a reference electrode connected to the aorta. The ECG was continuously monitored on a digital storage oscilloscope (model 1425,
Gould Electronic Ltd) and recorded on an ink-jet recorder (model RS3200, Gould Electronic Ltd). Chart speed was set at 50 mm/s a few seconds before reperfusion so a permanent high-speed record of the changes in the ECG during early reperfusion could be obtained. The ECG was retrospectively analysed, in a blinded manner, for the incidence, time-to-onset, and duration of ventricular tachycardia (VT) and ventricular fibrillation (VF). All analyses were carried out in accordance with the Lambeth Conventions.\textsuperscript{25} VT was defined as 4 or more consecutive premature beats of ventricular origin and VF as a signal in which both rate and amplitude varied from cycle to cycle.

Coronary Flow and Heart Rate Flow in each coronary bed was monitored throughout the experiment, with in-line flow detectors. Heart rate was determined from the ECG trace at selected intervals.

Heart Weight and Size of Ischemic Zone At the end of each experiment, the coronary bed subjected to ischemia was perfused at 75 mmHg for 30 s with a solution containing 0.02% disulphine blue dye. The heart was then removed from the perfusion apparatus, the atria and mediastinal tissue were removed, and the dye-stained tissue (representing ventricular myocardium subjected to ischemia) was carefully dissected out. The stained and unstained tissues were lightly blotted and weighed. LV (free wall and whole ventricular septum) and right ventricular (RV) (free wall) weights were expressed as the ratio of heart weight to body weight (g/kg). The size of the ischemic zone, expressed as a percentage of total ventricular weight, was calculated from the equation:

\[(\text{weight of stained or unstained tissue/total ventricular weight}) \times 100\]

The absolute weights obtained also enabled the calculation of flow in the left and right coronary beds on the basis of tissue weights supplied by each bed (ml·min\(^{-1}\)·g\(^{-1}\)).

Exclusion Criteria Exclusion criteria, selected to avoid cross-flow between the right and left coronary ostia following a mismatch between aorta size and cannula diameter, were based on our previous experience with the model.\textsuperscript{21} To verify whether significant cross-flow occurred at the beginning of each experiment, the perfusion line to one bed was clamped for a brief period (10 s) that in itself would not alter the subsequent responses to ischemia and reperfusion. If flow to the contralateral bed increased by more than 10% of the preceding flow in the occluded bed, the heart was excluded from the study (because collateral flow alone could not have been responsible for such an increase).\textsuperscript{26} In addition, hearts that exhibited ventricular arrhythmias during the final 3 s of ischemia before reperfusion were excluded from the analysis of reperfusion-induced arrhythmias, because in those hearts it would have been impossible to differentiate arrhythmias induced by reperfusion from those induced by ischemia. Of 62 hearts entered into the arrhythmia study, 1 was excluded on account of cross-flow and 1 on account of arrhythmia during the final 3 s of ischemia. The overall exclusion rate was 3%.

Plasma ACE Activity The inhibitory effect of ramipril on plasma ACE activity was determined in blood taken from randomly chosen animals in the arrhythmia study (n=6 per group). Blood was collected through the carotid artery cannula after the administration of heparin (200 IU) and the plasma was separated from cells by centrifugation at 10,000 G for 10 min (4°C) and kept at –20°C until enzyme assay. ACE activity was measured spectrophotometrically with a commercial kit (Sigma, Dorset, UK) according to the directions. Briefly, an aliquot (0.1 ml) of plasma was mixed with 1 ml of substrate (37°C). Decrease of absorbance at 340 nm was measured over a 5-min period, starting at 5 min after the addition of plasma (SP8-100UV Spectrophotometer, Pye Unicam Ltd, Cambridge, UK). ACE activity (IU/L) was determined by comparison with the reaction rate obtained with a calibration solution (supplied with the assay kit).

Hydroxyproline Content Left and right ventricles from the hearts of the SH, AC, AC+R0.01, AC+R0.1, and AC+R1 rats (n=6 per group) were frozen until use. The ventricles were thawed and hydrolysed in 2 ml of 6N HCl for 24 h at 100°C. After evaporation to dryness, the hydrolysates were resuspended in water, re-evaporated to dryness, and resuspended in 2.5 ml of 0.2 mol/L sodium citrate/0.2 mol/L sodium acetate at pH 6. Hydroxyproline content was measured as described previously.\textsuperscript{27} Results were expressed as total amount (mg) and concentration (mg/g wet or dry heart weight) of hydroxyproline in each ventricle. Thirty hearts entered into the hydroxyproline study and none was excluded.

Myocyte Size Left and right ventricular myocytes were isolated from hearts from a separate group of SH, AC, and AC+R1 hearts (n=3 per group) via a collagenase-based digestion technique.\textsuperscript{28,29} Aliquots of storage buffer containing myocytes were transferred with a pipette into a Petroff-Hauser bactria counting chamber and observed with x1,000 magnification. The image was displayed on a viewing screen, and the maximum width and length of individual myocytes (150 cells per ventricle) were measured with a ruler. Nine hearts entered into the myocyte size study and none was excluded.

mRNA Ratio of ANF/Glutamic Acid Decarboxylase (GAD) in Left Ventricle At the same time point, the heart was rapidly removed from separate groups of SH (n=6), AC (n=5), and AC+R1 (n=6 per group) rats after they were killed by cervical dislocation. The left ventricle was separated, immediately frozen in liquid nitrogen, and stored at –80°C. Total tissue RNA was extracted from each group by the LiCl/urea precipitation method and pooled for later analysis as required. RNA concentrations were determined by spectrophotometry at 260 nm. An aliquot of each RNA sample was electrophoresed on agarose gel and stained with ethidium bromide; those that were not degraded were subjected to reverse transcription polymerase chain reaction (RT-PCR). The mRNA ratio of ANF/GAD was measured with RT-PCR, which we used in our previous study.\textsuperscript{30} Eighteen rats entered into the ANF mRNA study and 1 animal in the AC group died 5 weeks after the surgery from cardiac failure.

Statistical Analysis All experiments were carried out in a prospectively randomized manner, with concurrent controls. Statistical analyses were based on the guidelines described by Wallenstein et al.\textsuperscript{31} Gaussian-distributed variables were expressed as mean±SEM and were subjected to one-way analysis of variance. If a difference among mean values was established, Student Newman Keuls test was used for inter-group comparisons. In the myocyte size study, inter-
group comparison was by Student t-test with Bonferroni correction for multiple comparisons. Binomially distributed variables, such as the incidence of VT or VF, were compared using the chi-square test for a 2×n table. If a significant difference was revealed, each AC group was compared with the SH group using the Fisher exact test. A value of p<0.05 was considered significant. Of 119 rats entered into the study, 3 were excluded for reasons described earlier. The overall exclusion rate was 2.5%.

Results

Effects of Ramipril on BP and Plasma ACE Activity

The effects of ramipril on arterial BP and plasma ACE activity are shown in Table 1. At 6 weeks after surgery, AC tended to increase plasma ACE activity, but there was no statistically significant difference between the AC and SH groups at that time point. Three weeks of ramipril treatment, however, decreased plasma ACE activity in a dose-dependent manner. At the same time point, AC significantly increased systolic BP in the carotid artery. Ramipril treatment lowered that increase in a dose-dependent manner such that BP at the 2 highest doses (AC+R0.01, AC+R0.1) was significantly less than that in the AC group, confirming the successful suppression of plasma ACE activity. Although femoral artery BP in the SH and AC groups was similar, ramipril treatment reduced it in a dose-dependent manner. The pressure gradient between the carotid and femoral arteries was significantly higher in the AC group than the SH group, confirming the efficacy of clipping the abdominal aorta, and ramipril treatment did not affect that gradient.

Effects of Ramipril on Heart Weight

The effects of ramipril on heart weight are shown in Table 1. Although there was no statistical difference in initial body weight between groups, the final body weight in the drug-treated groups was less than in the SH group. Consistent with our previous study, LV weight 6 weeks after surgery was significantly greater in the AC group than in the SH group. Ramipril treatment tended to decrease LV weight at the 2 highest doses, but there was no significant difference from LV weight in the AC group. The ratio of LV weight to body weight, again, was significantly greater in the AC group than in the SH group, and ramipril treatment did not affect that ratio at any dose used (the ratio in the treated group was still higher than the ratio in the SH group), a reflection of lower final body weight. RV weight was similar in all groups, both as a raw value and the RV
ischemia was 33%. AC increased the VF incidence to 92% (p<0.05 vs SH). Ramipril decreased the VF incidence to 42%, 50%, and 42% at doses of 0.01, 0.1, and 1 mg/kg per day, respectively, such that the incidences of VF were similar for the drug-treatment groups and the sham-operated control group. The reduction of VF incidence was not dose-dependent, and the decrease of the incidence relative to the AC group was statistically significant even at the lowest dose. In order to analyse the relation between VF incidence and LV weight/body weight ratio, animals with AC were divided into 2 groups: no-VF (n=21) and VF (n=27), as shown in Fig.3. Mean values of the ratio were 3.30±0.09 and 3.32±0.11 in the no-VF and VF groups, respectively (NS).

### Ischemic Zone Size, Coronary Flow and Heart Rate

Fig1 shows the time course of reperfusion-induced arrhythmias in all the groups. More than 67% of the hearts in every group developed VT within a few beats after the onset of reperfusion in the SH (8/12), AC (11/12), AC + R0.01 (11/12), AC + R0.1 (9/12) and AC + R1 (10/12) groups (differences not significant; NS). VT degenerated into VF in 4/8 cases in the SH group and all cases (11/11) in the AC group. Ramipril treatment tended to inhibit the degeneration of VT into VF to 5/11, 6/9 and 5/10 cases at 0.01, 0.1, and 1.0 mg/kg per day, respectively (NS). By the end of the 5-min reperfusion period, only 4/12* hearts in the SH group and 5/12*, 6/12 and 5/12* in the drug-treatment groups were in VF, and 11/12 hearts in the AC group were in VF (*p<0.05 vs AC). Once hearts degenerated to VF, they did not recover normal sinus rhythm regardless of drug treatment. Therefore, the inhibition of both the development of VT and degeneration of VT into VF (although neither of them showed statistical significance) contributed to a significant decrease in the VF incidence at the end of reperfusion in the ramipril treatment groups. Fig.2 summarizes the overall incidences of reperfusion-induced VT and VF. Consistent with our previous study32 the incidence of reperfusion-induced VF in the non-hypertrophied hearts in the SH control group subjected to 7 min of regional ischemia was 33%. AC increased the VF incidence to 92% (p<0.05 vs SH). Ramipril decreased the VF incidence to 42%, 50%, and 42% at doses of 0.01, 0.1, and 1 mg/kg per day, respectively, such that the incidences of VF were similar for the drug-treatment groups and the sham-operated control group. The reduction of VF incidence was not dose-dependent, and the decrease of the incidence relative to the AC group was statistically significant even at the lowest dose. In order to analyse the relation between VF incidence and LV weight/body weight ratio, animals with AC were divided into 2 groups: no-VF (n=21) and VF (n=27), as shown in Fig.3. Mean values of the ratio were 3.30±0.09 and 3.32±0.11 in the no-VF and VF groups, respectively (NS).

### Myocyte Size

Only the highest dose of ramipril (1 mg/kg per day) significantly increased LV hydroxyproline concentration based on dry weight, and that is the dose we studied for effect on myocyte size. The results are shown in Table 4.
Effects of Ramipril on Regression of Hypertrophy

Contrary to the results of some of earlier studies,16,17,33–36 treatment with ramipril (0.01–1 mg/kg per day) did not affect LV weight/body weight ratio in LV hypertrophy. The present data on plasma ACE activity and arterial BP clearly indicated that the animals ingested the expected doses of drug with their drinking water. The doses were selected from those used in earlier studies17,33–36 that caused a significant decrease in LV weight/body weight ratio; in those studies, ramipril significantly decreased LV mass at the lowest dose used in the present study (0.01 mg/kg per day) without affecting arterial BP, although longer treatments (6 weeks to 1 year) were necessary for the mass to decrease. Therefore, the reason the drug did not reduce hypertrophy in the present study is probably that we did not use it for a long enough period (3 weeks). In support of this, 2 studies37,38 showed that 2 or 3 weeks of ramipril treatment does not affect LV weight/body weight ratio, even at 1 mg/kg per day.

It should be noted that in the present study ramipril significantly decreased VF incidence even at the lowest dose, which did not affect LV weight or the LV weight/body weight ratio. That VF incidence did not vary with LV weight/body weight ratio in animals with LV hypertrophy. The present study shows that ramipril treatment decreased VF incidence even at the lowest dose (0.01 mg/kg per day), and that VF incidence did not vary with LV weight/body weight ratio in animals with LV hypertrophy."

Table 5 ANF/GAD mRNA Ratio in Sham-Operated Rats (SH), Rats With Aortic Constriction (AC) and Rats With Aortic Constriction That Were Given Ramipril (AC+R1)

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Mean ANF/GAD mRNA ratio</th>
</tr>
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<tbody>
<tr>
<td>SH</td>
<td>6</td>
</tr>
<tr>
<td>AC</td>
<td>5</td>
</tr>
<tr>
<td>AC+R1</td>
<td>6</td>
</tr>
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</table>

*p<0.05 vs SH

Aortic constriction caused significant increases in myocyte dimensions in both left and right ventricles. In left ventricular myocytes, both the long and short axes were significantly greater in the AC group than in the SH group. With ramipril treatment, the size of the short axis did not differ significantly between the SH and the AC+R1 groups, and the long axis in the AC+R1 group, though reduced, was still significantly greater than in the SH group. In right ventricular myocytes, aortic constriction increased only the long axis, and ramipril reversed the increase.

mRNA Ratio of ANF/GAD in the Left Ventricle

Ramipril (1 mg/kg per day) significantly increased the ratio of ANF mRNA to GAD mRNA in the LV. Its effects are shown in Table 5 (1 animal in the AC group died, probably from heart failure). The ratio of ANF mRNA to GAD mRNA in the hypertrophied LV was significantly greater in AC than in SH, and ramipril decreased that ratio to a value that did not differ significantly from the SH value.

Discussion

We have shown that ramipril treatment in the rat attenuates vulnerability to reperfusion-induced VF in LV pressure overload hypertrophy, even at a dose that affected neither arterial BP nor LV mass. The anti-arrhythmic effects of ramipril were equipotent at all the doses used in this study, in spite of the fact that the drug caused a dose-dependent decrease in plasma ACE activity and arterial BP. Ramipril did not affect the hypertrophied LV weight/body weight ratio at any dose used, but caused an increase in collagen content and a decrease in myocyte size in hypertrophied myocardium. Those changes were associated with an increased ratio of ANF mRNA to GAD mRNA in myocytes.
protocol, ramipril significantly altered both the dimensions and the developmental phenotype of myocytes in hypertrophied myocardium even without regression of LV mass. It also should be noted that the anti-arrhythmic effect of ramipril was equipotent at all doses employed in the present study despite the differences it induced in collagen quantity. Thus, our results indicate that intrinsic rather than geometric changes in hypertrophied myocytes may play an important role in the attenuation of the vulnerability to ventricular arrhythmias.

Ramipril significantly decreased myocyte size, although only the highest dose (1 mg/kg per day) was studied. Contrary to the results of myocyte size, ramipril treatment caused a dose-dependent increase in hydroxyproline concentration (which is used to assess the interstitial collagen types I and III, the most abundant components22). The disproportionate alterations in myocyte size and collagen quantity may reflect myocardial space previously occupied hypertrophied myocytes being replaced by reactively increased collagen.29 Such a reactive increase in collagen may explain why ramipril failed to reduce the LV weight. The increase in collagen quantity after the treatment with ACEI contrasts with the earlier studies15,16 and is probably because of the different treatment duration. Indeed, Pahor et al showed that long-term treatment (11 months) with the ACEI enarapril significantly decreased both LV weight and fibrous tissue fraction.15 The difference between their and a study with isolated rat hearts, one month after aortic banding, when the collagen network was not yet very pronounced, programmed electrical stimulation induced a significant increase in ventricular arrhythmias in the rats with hypertrophy.43 In the present study, ramipril increased myocardial collagen quantity in spite of the significant reduction of VF incidence. Those observations suggest that the effects of ACEI on myocardial fibrosis and arrhythmias are time dependent, and that the relationship between the extent of myocardial fibrosis and vulnerability to ventricular arrhythmias may differ among animal models. It is unlikely, at least in our model, that interstitial fibrosis played a significant role in arrhythmogenesis.

Myocyte hypertrophy is characterized by qualitative and quantitative changes in gene transcription.52-54 A transition occurs from the adult to the embryonic phenotype and involves up-regulation of β-myosin heavy chain and ANF genes44-46 which reverses during regression of hypertrophy and reversion to the adult phenotype.27,28 As in previous studies,47,48 the LV pressure overload in the present study significantly increased expression the ANF/GAD mRNA ratio in hypertrophied LV, and ramipril treatment reversed those changes. It has been shown that the embryonic phenotype is activated by protein kinase C (PKC)59 and Na+/H+ exchange51 and exaggerates vulnerability to reperfusion-induced arrhythmias.52 Although the role of Na+/H+ exchange and PKC activity in hypertrophied myocardium is still unclear, it is likely that, at least in our animal model, intrinsic changes such as reduction of PKC activity in myocytes play a major role in altering the vulnerability to arrhythmias during regression of hypertrophy. Further study is necessary, however, to determine the role of the dose dependency of ramipril in intrinsic changes such as the ANF mRNA level.

Possible Mechanisms Mediating the Anti-Arrhythmic Effects of Ramipril

In our previous study, brief (5 min) pretreatment of ischemic myocardium with ramiprilat (active moiety of ramipril) showed a protective effect on reperfusion-induced arrhythmias in normal rat heart.32 Bradykinin B2 receptor antagonist HOE140 reversed that effect, indicating that anti-arrhythmic effects are mediated through suppression of tissue ACE activity and a subsequent increase in endogenous bradykinin in ischemic myocardium.32 It has been shown that bradykinin mediates the anti-hypertrophic effects of ramipril (at the same doses used in the present study)13,40 and contributes to improvement of both metabolism and contractile function in hypertrophied myocardium41,42. That indicates that an increase in endogenous bradykinin may mediate the anti-arrhythmic effects of ramipril. The role of bradykinin in ischemia/reperfusion injury in hypertrophied myocardium, however, has never been studied, and it is unclear whether bradykinin contributed to the attenuation of vulnerability to reperfusion-induced VF in the present study. Contrary to ramipril’s significant anti-arrhythmic effect on hypertrophied heart in the present study, in our previous study, ramiprilat showed only limited anti-arrhythmic effect in the normal heart. This indicates that the anti-arrhythmic effect of the ACEI in the hypertrophied heart is mediated by something other rather than endogenous bradykinin. We did not, however, study the effect of ramiprilat administration to hypertrophied myocardium just before the ischemia. We are planning further study of ramiprilat and angiotensin II antagonist with the same animal model to confirm the role of bradykinin.

Pahor et al showed in spontaneously hypertensive rats that long-term treatment (11 months) with the ACEI enarapril significantly prevents the progression of hypertrophy and fibrosis and suppresses ventricular arrhythmias.53 They suggest that prevention of fibrosis diminishes the propensity to reentry cardiac remodelling and thus the incidence of ventricular arrhythmias. However, in another study with isolated rat hearts, one month after aortic banding, when the collagen network was not yet very pronounced, programmed electrical stimulation induced a significant increase in ventricular arrhythmias in the rats with hypertrophy.43 In the present study, ramipril increased myocardial collagen quantity in spite of the significant reduction of VF incidence. Those observations suggest that the effects of ACEI on myocardial fibrosis and arrhythmias are time dependent, and that the relationship between the extent of myocardial fibrosis and vulnerability to ventricular arrhythmias may differ among animal models. It is unlikely, at least in our model, that interstitial fibrosis played a significant role in arrhythmogenesis.

Clinical Relevance

Cardiac hypertrophy is a strong risk factor for cardiac sudden death from severe ventricular arrhythmias.1-5,53,54 ACEI are probably the most potent agents that can reverse cardiac hypertrophy41,54,55 and clinical trials have shown that they have anti-arrhythmic effects56,57 as well as reducing mortality58,59 in patients with congestive heart failure. Experimental studies have shown that regression of hypertrophy is strongly correlated with suppression of ventricular arrhythmias8,15,16 and improvement of survival rate48. Although differences in the hypertrophy model and the species studied should both be considered, our study indicates that regression of hypertrophy is not necessary for suppression of ventricular arrhythmias. The anti-arrhythmic effects of ramipril at the low doses used in the present study may be applicable for the treatment of conditions involving cardiac hypertrophy in the absence of hypertension, such as valvular disease and congenital heart disease.

In a previous study with the same animal model20 we measured cardiac output in vivo at the same time point used in the present study (6 weeks after the operation), but we found no significant difference between the sham-operated control group and the group with AC, suggesting that the
animal model was in a compensated state of hypertrophy. This indicates that cardiac hypertrophy is a potential risk factor even in a compensated state and suggests that drug treatment should be begun at an early stage of hypertrophy to prevent sudden death from ventricular arrhythmias.

**Study Limitations**

One limitation of this study was the deterioration of coronary reserve in the hypertrophied myocardium. Our recent study with the same model and the same doses of ramipril showed a significant decrease in coronary reserve in both right and left coronary beds in spite of drug treatment. In the present study, the flow rate was significantly lower in the left coronary bed than in the right coronary bed. Limiting the supply of perfusate to the hypertrophied myocardium during early reperfusion may affect myocardial injury during reperfusion. In this regard, in a previous study with hypertrophied neonatal rat heart, we observed a significant decrease in ventricular contractile function in hypertrophied hearts, and that was accompanied by a significant decrease in coronary flow rate during reperfusion. It should be noted, however, that our previous study with independent perfusion of the right and left coronary arteries showed that a decreased reperfusion flow rate does not affect the incidence of reperfusion-induced VT and VF, although we used normal non-hypertrophied rat hearts in that study.

It may be argued also that we cannot necessarily extrapolate from the rat heart, which has unusual electrophysiological characteristics (such as a short action potential duration and a high beating rate), to other species. While acknowledging this, it must be pointed out that the model offers many advantages in the study of the pathophysiological determinants of arrhythmogenesis, such as the consistent generation of regional ischemia and arrhythmias, the ability to use groups of adequate size (because of the low cost of preparation), and the ability to infuse drugs selectively into the ischemic/reperfused zone.

**Conclusion**

We studied the protective effect of ramipril upon reperfusion-induced arrhythmias using a LV hypertrophy model in rats with AC. Aortic constriction resulted in significant increases in arterial BP, LV weight/body weight ratio, and the incidence of reperfusion-induced VF. Three-week ramipril treatment decreased arterial BP and plasma ACE activity in a dose-dependent manner but failed to reverse the hypertrophied LV mass. It did, however, increase hydroxyproline quantity in a dose-dependent manner and significantly reduce hypertrophied myocyte dimensions, both of which were associated with reversion of the myocyte from the embryonic phenotype. Our results indicate that the anti-arrhythmic effects of ramipril are mediated by intrinsic changes in myocytes rather than by geometric alterations in the hypertrophied myocardium.

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