Left Ventricular Hypertrophy Decreases Slowly but Not Rapidly Activating Delayed Rectifier Potassium Currents of Epicardial and Endocardial Myocytes in Rabbits

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Background—Delayed rectifier K⁺ currents are critical to action potential (AP) repolarization. The present study examines the effects of left ventricular hypertrophy (LVH) on delayed rectifier K⁺ currents and their contribution to AP repolarization in both epicardial (Epi) and endocardial (Endo) myocytes.

Methods and Results—LVH was induced in rabbits by a 1-kidney removal, 1-kidney vascular clamping method. Slowly (I_{Ks}) and rapidly (I_{Kr}) activating delayed rectifier K⁺ currents were recorded by the whole-cell patch-clamp technique, and APs were recorded by the microelectrode technique. In normal rabbit left ventricular myocytes, I_{Ks} densities were larger in Epi than in Endo (1.1 ± 0.1 versus 0.43 ± 0.07 pA/pF), whereas I_{Kr} density was similar between Epi and Endo (0.31 ± 0.05 versus 0.36 ± 0.07 pA/pF) at 20 mV. LVH reduced I_{Ks} density to a similar extent (~40%) in both Epi and Endo but had no significant effect on I_{Kr} in either Epi or Endo. Consequently, I_{Kr} was expected to contribute more to AP repolarization in LVH than in control. This was confirmed by specific I_{Kr} block with dofetilide, which prolonged AP significantly more in LVH than in control (31 ± 3% versus 18 ± 2% in Epi; 53 ± 6% versus 32 ± 4% in Endo at 2 Hz). In contrast, L-768,673 (a specific I_{Kr} blocker) prolonged AP less in LVH than in control. The very small I_{Kr} density in Endo with LVH is consistent with the greater incidence of early afterdepolarizations induced in this region by dofetilide.

Conclusions—LVH induces a decrease in I_{Ks} density and increases the propensity to develop early afterdepolarizations, especially in Endo. (Circulation. 2001;103:1585-1590.)

Key Words: action potentials ■ hypertrophy ■ ion channels

Left ventricular hypertrophy (LVH) is associated with abnormal ventricular electrophysiology and increased risk of sudden cardiac death that is thought to be due to malignant ventricular arrhythmia. The action potentials (APs) of hypertrophied ventricular myocytes have long and nonuniform repolarization times. This dispersion of action potential duration (APD) is a milieu in which early afterdepolarizations (EADs) can generate sustained and highly disorganized repolarization times. The ionic mechanisms underlying the prolonged APD of hypertrophied myocytes are complicated.

Although the effects of LVH on L-type Ca²⁺ current (I_{Ca,L}), transient outward K⁺ current (I_{to}), and inward rectifier K⁺ current (I_{Ki}) have been extensively studied (for review, see Reference 1), LVH-induced modulation of the cardiac delayed rectifier K⁺ current (I_{K}) has received less attention despite its importance in determining ventricular repolarization and APDs, which are prolonged in LVH.¹⁻³ In feline right ventricular hypertrophy induced by pressure overload⁴ and LVH induced by aortic stenosis,⁵ total I_{K} density was decreased in hypertrophied myocytes, but the individual components, slowly (I_{Ks}) and rapidly (I_{Kr}) activating delayed rectifier K⁺ currents, were not discriminated. Therefore, it is unknown whether decreases in I_{K} density were due to changes in I_{Ks}, I_{Kr}, or both. In rabbit LVH induced by partial ligation of the abdominal aorta, I_{K} density was unchanged in hypertrophied myocytes compared with control myocytes, but I_{Ks} was not studied.⁶ Early studies of rabbit ventricular myocytes suggested that I_{K} in rabbit myocytes consists of only a single rapidly activating component.⁷⁻⁹ More recent studies, however, have demonstrated that both I_{Kr} and I_{Ks} are present in rabbit ventricular myocytes.¹⁰⁻¹²

In the LVH model of rabbits with renovascular hypertension, we previously demonstrated that 3 months after renal artery banding, the ventricular myocytes isolated from the middle layer of LV free wall are hypertrophied and have prolonged APD, decreased I_{Ca,L} density, increased I_{Ki} density, and unchanged I_{K1} density compared with control myocytes.⁷ The purpose of this study was (1) to characterize the effects of LVH on I_{Ks} and I_{Kr}, the 2 K⁺ currents critical to AP repolarization, and (2) to compare the effects of specific I_{Kr} or
I\textsubscript{Kr} inhibition on AP repolarization and incidence of EADs in ventricular myocytes isolated from normal and LVH rabbits. Because the heterogeneity of K\textsuperscript{+} channel distribution in ventricular myocardium has been associated with the physiological heterogeneity of APD across the ventricular wall,\textsuperscript{13–16} myocytes isolated from the epicardial (Epi) and endocardial (Endo) layers of LVs were studied separately.

**Methods**

**Experimental Animals**

Male New Zealand White rabbits (1.4 to 2.0 kg) underwent unilateral nephrectomy and contralateral renovascular banding to produce LVH by techniques reported previously.\textsuperscript{17} Banded rabbits were studied 3 months after surgery when documented LVH had developed. Data were collected from 24 control and 26 LVH rabbits.

**Myocyte Isolation**

Single ventricular myocytes were isolated by a method described previously.\textsuperscript{17} After enzyme perfusion, a thin layer (\(<1.5\) mm) of tissue was dissected from the Epi and Endo surfaces of the LV free wall, and myocytes were dispersed.

**Action Potential Recording**

AP was recorded at 36±0.3°C by the standard microelectrode technique. Microelectrodes had a resistance of 25 to 40 M\textohm; when filled with 3 mol/L KCl. Cells were superfused with a solution containing (in mmol/L) NaCl 137, KCl 5, MgCl\textsubscript{2} 1, CaCl\textsubscript{2} 2, glucose 10, and HEPES 10 (pH 7.4). AP was recorded at steady state with various stimulus frequencies (0.2, 0.5, and 2 Hz). Because EADs appeared in some of the APs recorded at 0.2 and 0.5 Hz under certain conditions, quantitative analysis of APD was performed only for the AP recorded at 2 Hz.

**Membrane Current Recording**

I\textsubscript{Kr} and I\textsubscript{Ks} were recorded at 36±0.5°C by the whole-cell patch-clamp technique. Because of the small amplitude of I\textsubscript{Kr} and I\textsubscript{Ks} in rabbit ventricular myocytes, the conditions used for I\textsubscript{Kr} and I\textsubscript{Ks} recording were different, to better resolve each component. Electrodes had a resistance of 25 to 40 M\textohm; when filled with 3 mol/L KCl. Cells were superfused with a solution containing (in mmol/L) NaCl 137, KCl 5, MgCl\textsubscript{2} 1, CaCl\textsubscript{2} 2, glucose 10, and HEPES 10 (pH 7.4). AP was recorded at steady state with various stimulus frequencies (0.2, 0.5, and 2 Hz). Because EADs appeared in some of the APs recorded at 0.2 and 0.5 Hz under certain conditions, quantitative analysis of APD was performed only for the AP recorded at 2 Hz.

**Data Analysis**

Data are expressed as mean±SEM. Cell numbers are indicated in parentheses in the table and figures. Statistical analyses were performed with GraphPad Prism 2.0 (GraphPad Software, Inc). Two-way ANOVA was used to compare data of Epi and Endo from control and LVH. Unpaired t test was used to examine statistically significant differences observed with the 2-way ANOVA. Contingency tables were used to compare EAD incidence between control and LVH. Fisher’s exact test was used for P value calculations.

**Results**

Three months after renal artery banding, the ratio of heart weight to body weight increased from 2.15±0.03 g/kg (n=24) in control to 2.63±0.06 g/kg (n=26) in LVH (P<0.05). Cell membrane capacitance of ventricular myocytes isolated from Epi and Endo increased 23% and 29%, respectively, in LVH compared with control (Table). APD measured at 60% and 90% repolarization (APD\textsubscript{60} and APD\textsubscript{90}) at 2 Hz was significantly prolonged in both Epi and Endo from LVH rabbits compared with controls (Table).

The isolation of I\textsubscript{Kr} is demonstrated in Figure 1A. I\textsubscript{Kr} current-voltage relations for control and LVH are compared in Figure 1B for Epi and Endo. LVH significantly decreased I\textsubscript{Kr} density in both Epi and Endo. The isochronal activation curves for I\textsubscript{Kr} were essentially identical between Epi and Endo for a given condition; therefore, regional data were averaged, and those results were plotted for the control and LVH conditions in Figure 1C.

Example I\textsubscript{Kr} traces at \(V_s\) from −30 to +20 mV are shown in Figure 2A. I\textsubscript{Kr} current-voltage relations for control and LVH are compared in Figure 2B for Epi and Endo. I\textsubscript{Kr} density was unchanged in LVH compared with control in either Epi or Endo. The voltage-dependent activation of I\textsubscript{Kr} was compared between control and LVH myocytes in Figure 3 for Epi...
and Endo. The voltage-dependence of activation of $I_{Ks}$ was essentially identical for LVH and control myocytes. The effects of L-768,673 and dofetilide on AP were examined to determine the relative contribution of $I_{Ks}$ or $I_{Kr}$ to AP repolarization in control and LVH rabbits. AP example recordings at 2 Hz are shown for myocytes from Epi and Endo of normal and LVH in Figures 4 and 5, and averaged percent increases in APD90 induced by drugs are summarized in Figure 6. APD is typically shorter in Epi than in Endo and is universally prolonged by LVH (Table). Inhibition of $I_{Ks}$ with 0.1 μmol/L L-768,673, a concentration expected to produce nearly total $I_{Ks}$ block, produced modest increases in APD (Figure 4). APD prolongation was greater in Epi than in Endo and notably, was significantly less for LVH than control (Figure 6). In contrast, inhibition of $I_{Kr}$ with 0.1 μmol/L dofetilide, a concentration expected to produce nearly total $I_{Kr}$ block, caused a completely opposite pattern of APD changes, which were also remarkably greater than those observed with $I_{Ks}$ inhibition (Figure 5). APD was increased more in Endo than in Epi and more importantly, was prolonged more for LVH than control (Figure 6).

At a very low stimulus frequency of 0.2 Hz, spontaneous EAD was observed in 2 of 12 myocytes from Epi and 5 of 21 from Endo of LVH. Figure 7A shows an example trace of a spontaneously occurring EAD recorded from an LVH Endo cell. In contrast, spontaneous EAD was never observed in control (0.2 Hz) or in LVH at stimulus frequencies $\geq 0.5$ Hz. Notably, L-768,673 (0.1 μmol/L) did not induce EAD in Epi or Endo of either control or LVH (cell number $\geq 12$ for each condition). In contrast, dofetilide (0.1 μmol/L) induced EAD in almost all LVH Endo myocytes, and although it prolonged APD in control Epi, it did not induce EAD (Figure 7B). At 0.5 Hz, the incidence of EAD induced by 0.1 μmol/L.
dofetilide was 0 of 15 cells in Epi and 4 of 13 cells in Endo for control, compared with 3 of 12 cells in Epi and 11 of 12 cells in Endo for LVH. EAD incidence in Endo induced by 0.1 μmol/L dofetilide was significant higher in LVH than control (P<0.05).

Discussion

AP Prolongation in Hypertrophied Epi and Endo

LVH was induced consistently in rabbits 3 months after renal artery banding, as confirmed by significant increases in the ratio of heart weight to body weight. Hypertrophy occurred in both Epi and Endo, as demonstrated by the significant increases in cell membrane capacitance of myocytes from both LV layers. In control rabbits, APD was significantly longer in Endo than in Epi, as has been found in other animal species.15,22,23 In the present study in rabbits, we have shown that this gradient or dispersion of APD is at least partially due to a smaller \(I_{Ks}\) density in Endo than in Epi. Moreover, in LVH rabbits with renal artery banding, we have also shown that prolongation of APD was similar in Epi and Endo compared with control rabbits. APD at 2 Hz was increased by 24% in Epi and 22% in Endo. This result differs from findings in another rabbit study of LVH produced with perinephritis-induced hypertension, in which APD prolongation was more pronounced in Epi than in Endo.2 The underlying ionic mechanism for more pronounced AP prolongation in Epi than Endo in this model was not given. We also found that the average APD of Endo was the same as that of midlayer myocytes in both control and LVH rabbits.3

LVH Decreases \(I_{Ks}\) but Not \(I_{Kr}\) Density

Pathology-induced modulation of \(I_{Ks}\) and \(I_{Kr}\) varies with animal model. In LVH rabbits with renovascular hypertension, we found an \(\sim 40\%\) decrease of \(I_{Ks}\) density in both Epi and Endo but no significant change of \(I_{Kr}\) density (Table). In another rabbit study, in which LVH was induced by partial ligation of the abdominal aorta, \(I_{Ks}\) density was also found to be unchanged in hypertrophied myocytes compared with controls; however, the effect of LVH on \(I_{Kr}\) was not studied.6 In a guinea pig model with aortic banding, \(I_{Ks}\) and \(I_{Kr}\) densities remain unchanged during cardiac hypertrophy and failure.24 In midmyocardial cells of dogs with chronic complete atrioventricular block, \(I_{Ks}\) density decreased in both left and right ventricles, whereas \(I_{Kr}\) density decreased in the right ventricle only.25 The renovascular hypertension model of rabbits we
used is similar to essential hypertension in humans in that this model produces gradual hypertrophy in the appropriate ventricle.

Voltage-Dependent Activation of \( I_{Ks} \) and \( I_{Kr} \)

\( I_{Kr} \) recorded from rabbit LV cells in this study (Figure 2B) displayed a typical bell-shaped current-voltage relationship, similar to that of guinea pig ventricular myocytes, rabbit sinoatrial node cells, and ferret atrial myocytes. This pronounced inward rectification of \( I_{Kr} \) is due to its unique rapid inactivation, which occurs more quickly than activation at more depolarized potentials. 

Voltage-dependence of activation for \( I_{Ks} \) or \( I_{Kr} \) was not different between Epi and Endo of control or LVH rabbits. LVH had no effect on the voltage-dependence of activation of either \( I_{Ks} \) or \( I_{Kr} \). Similar to our findings, chronic complete atrioventricular block in dogs has no effect on the voltage-dependence of activation of \( I_{Ks} \) or \( I_{Kr} \). The steady-state activation of total \( I_{K} \) does not differ between normal and hypertrophied myocytes in feline right ventricular hypertrophy.

Differential Modulation of AP by \( I_{Ks} \) or \( I_{Kr} \) Block

In control rabbits, Epi had significantly larger \( I_{Ks} \) density than Endo, whereas both layers had similar \( I_{Kr} \) density. Therefore, \( I_{Kr} \) was expected to contribute more to AP repolarization in Epi than Endo, whereas \( I_{Ks} \) would contribute more to AP repolarization in Endo than Epi. Consistent with this argument, we found that in control rabbits, the selective \( I_{Ks} \) blocker L-768,673 induced larger increases of APD\(_{90}\) in Epi than Endo (21 ± 3% versus 8.8 ± 1.3%, \( P < 0.05 \)), whereas the selective \( I_{Kr} \) blocker dofetilide induced greater prolongation of AP in Endo than Epi (32 ± 4% versus 18 ± 2%, \( P < 0.05 \)).

In LVH rabbits, because of a decreased \( I_{Kr} \) density and an unchanged \( I_{Ks} \) density in both Epi and Endo, the effects of \( I_{Kr} \) block on AP repolarization were significantly reduced in LVH. L-768,673 induced significantly smaller percentage increases of APD\(_{90}\) in both Epi (10 ± 1% versus 21 ± 3%) and Endo (5.1 ± 0.6% versus 8.8 ± 1.3%) in LVH rabbits compared with controls. Conversely, the decreased \( I_{Ks} \) in both Epi and Endo of hypertrophied myocytes made \( I_{Ks} \) more critical to AP repolarization in LVH rabbits. Blocking \( I_{Ks} \) with dofetilide induced significantly larger prolongation of AP in both Epi (31 ± 3% versus 18 ± 2%) and Endo (53 ± 6% versus 32 ± 4%) of LVH rabbits compared with controls at 2 Hz.

Spontaneous and Drug-Induced EAD

Among the 4 groups of myocytes that we studied, Endo myocytes from LVH rabbits had the smallest \( I_{Ks} \) density (Table). At 0.2 Hz, spontaneous EAD was observed in 5 of 21 Endo cells and 2 of 12 Epi cells of LVH rabbits. Spontaneous EAD was not observed in any of the myocytes of control rabbits we examined. Whereas block of \( I_{Ks} \) by L-768,673 failed to induce EAD, block of \( I_{Kr} \) by dofetilide commonly led to EAD, especially in Endo of LVH rabbits. These observations may have important clinical implications, although the potential proarrhythmic effect of dofetilide would be reduced in vivo because of high heart rate and electrical coupling of myocardial tissue. It is now well recognized that agents that are selective blockers of \( I_{Ks} \) have the potential to produce excessive APD prolongation, leading to a prolonged QT interval. In extreme instances, they can cause EAD, which may underlie torsade de points arrhythmias observed with these agents clinically. Insofar as the changes observed in this study in rabbits are transferable to human cardiac hypertrophy and heart failure, our findings suggest that \( I_{Ks} \) block in this diseased state could even be more proarrhythmic in this patient population. In light of these recent observations, controversy has arisen as to whether any class III action, ie, increase in APD or cardiac refractoriness, could provide antiarrhythmic efficacy safely. Unlike the excessive increases in APD especially at slow heart rates, however, the limited increases observed with \( I_{Ks} \) block in this and other studies merit further study and consideration for its antiarrhythmic or proarrhythmic potential. Under the conditions of this study, there was no EAD induction even in the LVH rabbits with \( I_{Ks} \) block, raising the question of whether a controlled or limited increase in cardiac refractoriness could provide antiarrhythmic action by prevention of classic reentry. On the contrary, the reduction of the repolarizing current \( I_{Kr} \) with LVH, which itself could be considered proarrhythmic and contributing to the increased incidence of arrhythmias in this patient population, raises a converse hypothesis: namely, could an agent that increases a repolarizing current be of use in reversing or preventing increases in APD and arrhythmias and other sequelae of heart failure? One such agent that enhances \( I_{Kr} \) was described recently. Other studies have attempted to address this issue by using adenovirus-induced transfection to increase \( K^+ \) channel expression and augment repolarizing \( K^+ \) currents.

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References


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