Reduced Subendocardial Ryanodine Receptors and Consequent Effects on Cardiac Function in Conscious Dogs With Left Ventricular Hypertrophy

Luc Hittinger, Bijan Ghaleh, Jie Chen, John G. Edwards, Raymond K. Kudej, Mitsunori Iwase, Song-Jung Kim, Stephen F. Vatner, Dorothy E. Vatner

Abstract—The goal of this study was to examine the transmural distribution of ryanodine receptors in left ventricular (LV) hypertrophy (LVH) and its in vivo consequences. Dogs were chronically instrumented with an LV pressure gauge, ultrasonic crystals for measurement of LV internal diameter and wall thickness, and a left circumflex coronary blood flow velocity transducer. Severe LVH was induced by chronic banding of the aorta (12 ± 1 months), which resulted in a 78% increase in LV/body weight. When ryanodine was infused directly into the circumflex coronary artery, it did not affect LV global function or systemic hemodynamics; however, it reduced LV wall thickening and delayed relaxation in the posterior wall in control dogs but was relatively ineffective in dogs with LVH. In LV sarcolemmal preparations, [3H]ryanodine ligand binding revealed a subendocardial/subepicardial gradient in normal dogs. In LVH there was a 45% decrease in ryanodine receptor binding and a loss in the natural subendocardial/subepicardial gradient, which roughly correlated inversely with the extent of LVH and directly with regional wall motion. Both mRNA and Western analyses revealed similar findings, with a reduction of the transmural mRNA levels and a loss in the natural gradient between subendocardial and subepicardial layers in LVH. Thus, ryanodine receptor message and binding in LVH is reduced preferentially in the subendocardium with consequent attenuation of the action of ryanodine in vivo. The selectively altered ryanodine regulation subendocardially in LVH could reconcile some of the controversy in this field and may play a role in mediating decompensation from stable LVH. (Circ Res. 1999;84:999-1006.)

Key Words: pressure-overload hypertrophy ▪ systole ▪ diastole ▪ Ca^{2+} ▪ sarcoplasmic reticulum

The ryanodine receptor plays a critical role in excitation-contraction coupling. Several studies have reported that administration of ryanodine depresses both systolic and diastolic cardiac function in the normal heart.1–6 In left ventricular (LV) hypertrophy (LVH) and in heart failure, the regulation of cardiac function by the ryanodine receptor is controversial. Biochemical studies have suggested that ryanodine receptor density is increased,7 is not changed,8 or is reduced9–11 in hypertrophy. Prior studies have reported that levels of ryanodine receptor mRNA are increased in mild hypertrophy but decreased in severe hypertrophy.12,13 In mild hypertrophy, sarcoplasmic reticulum (SR) Ca^{2+} transport is also increased,14 and in human heart failure, single-channel recordings are normal.15 In heart failure, which usually includes a component of hypertrophy, ryanodine receptor binding may be increased,16 not changed17,18 or decreased.19,20 Studies examining protein levels of the ryanodine receptor in heart failure have shown no change,16,21,22 but other studies have shown that mRNA levels are decreased.16,19,23 Furthermore, studies have shown selective decreases in ryanodine receptor mRNA in ischemic cardiomyopathy compared with dilated cardiomyopathy24 or decreases in both ischemia and dilated cardiomyopathy.25 Importantly, prior studies have not directly examined the effects of ryanodine on global and regional myocardial function in LVH, and biochemical studies have not focused on regional aspects but only provide transmural data. This latter aspect is critical in view of our hypothesis that there is altered transmural distribution of ryanodine receptors in LVH, potentially related to major transmural differences in blood flow regulation in LVH.26,27 To reconcile this controversy, it would be important to obtain in the same study functional data in vivo in combination with biochemical evidence of altered transmural ryanodine receptor distribution in the heart.

To address this question for the first time, a large mammalian model was used that permits this type of investigation.28 The first goal of the current investigation was to...
determine the direct effects of ryanodine on systolic and diastolic LV function in conscious dogs with severe LVH. To avoid the effects of LV ryanodine on LV loading conditions, experiments were performed with ryanodine delivered into the coronary artery, where effects on preload and afterload do not complicate the interpretation of ryanodine administration. It was particularly important to study conscious dogs, since anesthesia can also affect excitation-contraction coupling. The second goal of the current investigation was to determine the density and the affinity of ryanodine receptors in severely hypertrophied hearts and whether these changes are affected transmurally in LVH. The third goal was to determine whether the altered regulation of ryanodine receptors was posttranslational or due to altered gene expression.

Materials and Methods

Development of Model

Mongrel puppies of either sex at 8 to 10 weeks of age were anesthetized with 12.5 mg/kg sodium thiopental, maintained with halothane anesthesia (1 to 2 vol %), and ventilated with a respirator (Harvard Apparatus). A right thoracotomy was performed through the fourth intercostal space by use of a sterile surgical technique. The ascending aorta above the coronary arteries was isolated and dissected free of surrounding tissue. A 1-cm-wide polytetrafluoroethylene (Teflon) cuff was placed around the aorta and tightened until a thrill was palpable over the aortic arch; then the chest was closed. The Teflon band created a fixed supraavalvular aortic lesion, which became relatively more stenotic as the puppies grew. The puppies were followed for 8 to 19 months.

Implantation of Instrumentation

Two protocols were used in this investigation. Ryanodine was administered intracoronarily to 6 control and 5 LVH dogs. In vitro studies depended on an availability of both epicardial and endocardial LV samples and accordingly were performed on a subset of these dogs and on samples from prior experiments in which ryanodine had been infused intravenously. After induction with sodium thiopental (12.5 mg/kg) and maintenance with halothane anesthesia (1 to 2 vol %), an incision was made in the fifth left intercostal space by use of a sterile surgical technique. All dogs were instrumented with Tygon catheters (Norton Elastics and Synthetic Division) implanted in the descending thoracic aorta and left atrium and in the LV apex for the aortic-banded dogs. A solid-state miniature pressure transducer (model P22, Konigsberg Instruments) was implanted in the left ventricle through the apex to measure LV pressure in all dogs. LV wall thickness was measured with piezoelectric crystals implanted across the anterior and posterior walls. The left circumflex coronary artery was isolated 3 to 5 cm from its origin, and an ultrasonic Doppler blood flow transducer was implanted around the vessel in control dogs and dogs with LVH. An indwelling silastic catheter was implanted in the left circumflex coronary artery and in the coronary sinus. The thoracotomy incision was closed in layers, and the animals were allowed to recover for 2 weeks before the study. The animals used in this study were maintained in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, revised 1996).

Experimental Protocol

Experiments were performed in a quiet laboratory with the undated, conscious dogs resting comfortably in the right lateral position. A stock solution of 5 mg/mL ryanodine (Calbiochem) was prepared in distilled water and stored in the freezer at −20°C. Solutions for intracoronary administration were freshly prepared on the day of the experiment by dilution of the stock solution with 0.9% NaCl solution as necessary. The effects of cumulative intracoronary doses of ryanodine (0.5, 1, 3, and 6 µg) were examined in the 5 dogs with LVH and the 6 control dogs. Ryanodine was infused directly into the circumflex coronary at a rate of 1 mL/min. A 5-minute period for stabilization of the effects of ryanodine was allowed between each dose.

The data were recorded on a multichannel tape recorder (Honeywell) and played back, and measurements were made using Hem 1.4 (Notocord Systems) on a Vectra XU 5/133C Hewlett Packard computer. LV end-diastolic wall thickness was measured at the outset of LV contraction, indicated by the initial increase in LV dP/dt. LV end systole was defined as the point of maximum negative inflection of LV dP/dt. To assess initial changes in relaxation during intracoronary administration of ryanodine, the slope of the LV posterior wall motion during the isovolumic relaxation phase was computed. Normally, during isovolumic relaxation, the wall begins to thin and the slope is negative. However, when diastolic relaxation is delayed or inhibited, the slope becomes less negative or even positive. LV systolic function was assessed by examining the extent of systolic wall thickening.

Membrane Preparation

After the dogs were anesthetized with sodium pentobarbital (40 to 50 mg/kg), their hearts were excised immediately and placed in iced saline. The left ventricle and septum were weighed, and samples from the LV free wall were divided into half into subendocardial and subepicardial layers and trimmed of fat and connective tissue, and a crude membrane fraction was prepared as previously described. It must be recognized that some ryanodine receptors are lost in the first centrifugation. This was quantified in heart samples from 3 dogs by measuring ryanodine receptors in the crude preparation before the first centrifugation and in the supernatant after the membrane fraction was prepared. The fraction of ryanodine receptors in supernatant from subendocardial and subepicardial layers was similar.

Receptor Binding Studies

To optimize the binding conditions for [3H]ryanodine binding, 12 concentrations of membrane protein (10 to 200 µg) were assayed with and without unlabeled ryanodine (10 µmol/L) and with 50 nmol/L [3H]ryanodine (61.5 Ci/mmol). Incubation was at 37°C for 2 hours. All assays were performed in triplicate and terminated by rapid filtration through Whatman glass fiber filters washed with 4 mL of cold buffer (150 mmol/L KCl and 10 mmol/L Tris-HCl, pH 7.4). Filters were vortexed in 5 mL of Ecoscint and counted in a beta scintillation counter for 5 minutes. Specific binding with 70 µg of protein was >90%. The optimal time for incubation when the reaction reached equilibrium was determined by incubating 70 µg of membrane protein and 8 mmol/L [3H]ryanodine for increasing amounts of time from 1 to 120 minutes at 37°C. The samples were filtered and counted. Optimal calcium concentration was determined by incubating 70 µg of membrane protein with 5 nmol/L [3H]ryanodine in HEPES buffer containing (in mmol/L) HEPES 10, EGTA 1, KCl 150, and AMP 3 (pH 7.4) with increasing concentrations of CaCl2 (1 µmol/L to 10 mmol/L). Incubation was carried out at 37°C for 2 hours.

Ryanodine binding sites were quantified with 8 concentrations of [3H]ryanodine (1 to 40 nmol/L) and 100 µg of membrane protein, in the presence and absence of 10 µmol/L (unlabeled) ryanodine in a final volume of 150 µL. The tissue was prepared in HEPES buffer with 2 mmol/L CaCl2. The triplicate samples were incubated for 2 hours at 37°C, filtered on Whatman glass fiber filters with 4 mL of wash buffer 3 times for each sample (10 mmol/L Tris-HCl and
Effects of Intracoronary Ryanodine on Global and Regional Myocardial Function

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<th>Baseline</th>
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Values are mean±SEM.

*P<0.05 LVH different from control.
†P<0.05 effects of ryanodine different for baseline.

Western Analysis

The amount of ryanodine receptor protein in cardiac membranes was measured by immunoblotting and run on precast 4% to 15% SDS-PAGE (Bio-Rad Laboratories). Proteins were then transferred to nitrocellulose paper using a wet transblotting apparatus for 4 hours at 1 A/cm². The blotting membrane was then incubated in Tris-buffered saline–Tween (TBST; 100 mmol/L NaCl, 100 mmol/L Tris-HCl, 0.1% Tween-20, and 5% nonfat dry milk) for 1 hour with shaking at room temperature. This was followed by a 1-hour incubation at room temperature in TBST, and the paper was then incubated for 20 minutes at room temperature with sheep anti-mouse horseradish peroxidase antibody (1:1000 dilution) and washed 4 times with TBST without milk. Autoradiography was performed using the chemiluminescence system (Amersham Corp) at room temperature for 1 minute. Autoradiographic densities were determined by densitometry (model PD, Molecular Dynamics).

mRNA Analysis

Changes in cardiac ryanodine receptors were determined by slot-blot analysis. LV total RNA was prepared and quantified as described previously.35 A cDNA coding for cardiac ryanodine receptor protein (cRYR2) was the generous gift of Dr P. Allen.36 Total RNA (10 µg) was slotted using a manifold (model PR 648, Hoefer, Pharmacia Biotech). The ryanodine cDNA was radiolabeled by a random prime method, and blots were normalized for loading using a 28S oligonucleotide (Clonetech) that was radiolabeled using the Prime-a-Gene Labeling system (Promega). Hybridization conditions were as previously described.35 After washing, the blots were placed under film at −80°C or in phosphor image cassettes. Autoradiographs or phosphor images were quantified using a densitometer or Storm 840 PhosphorImager, respectively (both from Molecular Dynamics).

Statistical Analysis

Statistical analysis was performed using StatView and SuperAnova software (Abacus Concepts Inc) on a Macintosh computer. The data are reported as mean±SEM. The comparison of the variations among groups were performed using the Student t test. The comparisons between groups and between baseline and responses were analyzed by a 2-way ANOVA for repeated measures, followed if necessary by a Student t test or a paired t test. Significance was recorded for probability value of 0.05 or less.

Results

Chronic pressure overload induced by aortic banding increased LV weight/body weight ratio by 78% (P<0.01) in
dogs with LV hypertrophy compared with control dogs. Right ventricular weights were similar in both groups.

Baseline Hemodynamics
Baseline hemodynamics are included in the Table. LV systolic pressure was doubled in dogs with LVH, but LV end-diastolic pressure, LV dP/dt, heart rate, and mean arterial pressure were similar in dogs with LVH and control dogs. LV end-diastolic wall thickness was 66% greater than in control dogs, reflecting the hypertrophy of the myocardial wall (Table).

Effects of Intracoronary Ryanodine
A typical example of the effects of intracoronary ryanodine in one of the control dogs is shown in Figure 1. Intracoronary ryanodine did not alter LV global function, systemic hemodynamics, or coronary blood flow velocity (Table). At a dose of 1 µg IC, ryanodine decreased LV posterior wall thickening (–18.1±2.5%, P<0.05) in control dogs (Figure 1 middle panel) but did not affect wall thickening in dogs with LVH (Figure 2, right panel). A clear dose-response relationship was observed in control dogs, whereas dogs with LVH demonstrated less depression of contraction in response to intracoronary ryanodine at any dose studied (Figure 2). At the highest dose of ryanodine studied, 6 µg, posterior LV wall thickening fell slightly (–8.9±2.6%) in dogs with LVH but much more (P<0.05) in control dogs (–42.0±5.6%). Intracoronary ryanodine delayed relaxation of the posterior LV wall more in the control dogs than in dogs with LVH. Intracoronary ryanodine replaced the normal wall thinning during isovolumic relaxation with a prolonged thickening, as illustrated in Figure 1. To account for potential dilutional effects in the larger hearts with dogs with LVH, the effects of 3 times the dose of ryanodine was compared in dogs with LVH (3 µg IC) versus control dogs (1 µg) in the Table. At 1 µg of ryanodine, changes in LV wall thickening and in the slope of the motion of the posterior LV wall thickness during isovolumic relaxation after intracoronary ryanodine were greater (P<0.05) in control dogs than with 3 µg in dogs with LVH, suggesting that ryanodine impaired local contraction and relaxation more in control dogs than in dogs with LVH. Neither coronary blood flow velocity (Table) nor coronary sinus oxygen content (data not shown) changed in both groups.

Ryanodine Receptors
Ryanodine binding studies for control and LVH hearts are shown in Figure 3. Figure 3 illustrates Scatchard analyses of compiled data from 13 animals, including 5 controls and 8 LVH hearts. There was a significant (P<0.01) transmural decrease in the amount of ryanodine receptor binding in LVH hearts with LV hypertrophy compared with control dogs. Right ventricular weights were similar in both groups.
hearts, with no significant change in the dissociation constant ($K_D$). Figure 4 shows that ryanodine receptor density for each animal studied was correlated with LV weight/body weight ($r=0.860$) (inverse) and LV wall thickening ($r=0.720$) (direct). Regional distribution analysis demonstrated a gradient of ryanodine receptor density between the subendocardial and subepicardial layers in control dogs. This was not observed in dogs with LVH (Figures 3 and 5, left panel). Western analysis confirmed this finding by also showing a loss in the natural subendocardial/subepicardial gradient in LVH.

**mRNA Analysis**

By Northern analysis, preliminary experiments determined that the cRYR2 probe produced only a single band of $\approx 16$ kb (data not shown). Because of the size of the ryanodine mRNA, efficient transfer did not always appear quantitative, and therefore slot-blot analysis was used to make comparisons between control and experimental animals. Compared with control animals ($n=5$), transmural LV cardiac ryanodine receptor mRNA levels were significantly decreased in LVH ($n=8$). When regional analysis was performed, a gradient in mRNA levels of ryanodine was observed between the subendocardial and subepicardial layers in control dogs. This gradient was not observed in LVH dogs (Figures 5, right panel, and 6).

**Discussion**

The effects of LV hypertrophy and heart failure on ryanodine regulation remains controversial. It is unlikely that this amount of controversy can be due to one factor. Prior studies have found both decreased$^9$–$^{11}$ and unchanged$^{11}$ ryanodine receptor density, whereas Limas et al$^{14}$ found enhanced calcium transport by the SR. This discrepancy may be explained by (1) methodological considerations (ie, measurement of ryanodine receptor binding$^9$–$^{11}$ versus mRNA level measurements)$^{12}$; (2) species differences, with the downregulation of ryanodine receptors appearing less marked in rats than in guinea pig or ferret$^{11}$; and (3) potentially, the duration or extent of LV hypertrophy, with upregulation of the ryanodine receptor being observed in mild hypertrophy or during the development of hypertension$^{12,13}$ and downregulation occurring after prolonged overload in severe hypertrophy.$^9$–$^{11}$ One other factor has not been considered, but may help reconcile this controversy, ie, the transmural distribution of changes. For example, if changes in ryanodine regulation occur selectively in one part of the heart, prior studies sampling nonselectively could have concluded no change if this was not taken into account and samples were diluted with tissues from regions where ryanodine regulation was not altered. The present study performed in dogs with severe LVH after prolonged pressure overload demonstrated clearly that ryanodine receptors are downregulated, but this downregulation is selective for the subendocardium. Furthermore, the decreased density of the ryanodine receptors correlates with the extent of hypertrophy and the baseline LV wall thickening (Figure 4).

Importantly, the current study demonstrated for the first time that there is a normal transmural gradient of ryanodine receptors, which is lost in LVH. The selective loss of ryanodine receptor regulation subendocardially may help to reconcile some of the controversy in this area. For example, prior negative studies may have been affected by subepicard-
Ryanodine in LV Hypertrophy

dial sampling, which does not show marked changes. The normal gradient from the endocardium to the epicardium for ryanodine receptors may be required to provide calcium for contraction in subendocardial layers, characterized by larger compressive forces. With the development of severe LVH, there is a loss of this natural transmural gradient of the ryanodine receptors between the subendocardial and subepicardial layers. Although the reason for the natural transmural gradient in the normal situation or its modification in LVH is not known, it is interesting to speculate that impairment in subendocardial ryanodine receptors is due to the subendocardial ischemia that develops on stress in LVH because of reduced subendocardial reserve.28,36 Interestingly, the deficit only becomes apparent with severe LVH, when restricted subendocardial coronary reserve is expressed. Furthermore, in patients with ischemic cardiomyopathy, downregulation of ryanodine receptor mRNA is observed.24 In addition, several studies have shown that acute myocardial ischemia can also reduce ryanodine receptors.37–39

A natural question is whether the downregulation of ryanodine receptors observed in LVH has to do with altered gene expression for the receptors or some other posttranslational event. It appears that the former alternative is responsible, as reflected by decreased levels of mRNA in the subendocardium in LVH. Interestingly, these experiments also demonstrated a transmural gradient for ryanodine mRNA in normal dogs and a loss of this transmural gradient in LVH. These experiments suggest that the key role of subendocardial reserve and subendocardial ischemia, which is so critical in mediating the transition from LVH to failure, may be part of the mechanism for reduced ryanodine receptors. This further links deficits in ryanodine binding to the development of heart failure, particularly ischemic cardiomyopathy, as already noted.19,20,24

It is also known that remodeling occurs with severe LVH, and there is increased fibrosis in subendocardial layers,27 which could decrease ryanodine message and receptors. However, in this model, the increase in fibrosis accounts for <2% of the subendocardial mass.27 Thus, a dilutional effect due to fibrosis was not responsible for the findings.

Although the observation that there is a downregulation of ryanodine receptors in severe LVH is important, little is known regarding the impact of this and the functional consequences. A major part of the current investigation was designed to examine the effects of ryanodine on LV function in LVH. The effects of ryanodine have been studied repeatedly in normal hearts and have consistently shown a dose-dependent decrease in LV systolic function.1–5 At the nanomolar concentration range, a range of concentrations produced by the doses of ryanodine given here,6 ryanodine binds to a high-affinity site, locking the channel in the semiopen state, resulting in calcium efflux and SR calcium depletion and finally a prolongation of the time course of LV contraction and relaxation.8 Since, in conscious dogs, ryanodine intravenously administered increases heart rate and changes the loading conditions2 and therefore alters systolic and diastolic function indices, in the current investigation ryanodine was delivered intracoronarily. Under these conditions, ryanodine did not elicit a major effect on global LV or systemic hemodynamics and coronary blood flow velocity. However, intracoronary ryanodine decreased systolic wall thickening more at each dose studied in control dogs than in dogs with LVH.

It is also well known that the calcium-release channel also regulates diastolic cardiac function. Several prior studies both in vitro4,40 and in vivo2,3 have demonstrated dose-dependent decreases in LV systolic and diastolic function with ryanodine administered systemically. This was confirmed in the present study in control dogs. However, we were surprised to find that the action of intravenous ryanodine was attenuated strikingly in LVH (data not shown). It is important to keep in mind, however, that changes in loading conditions alter the interpretation of diastolic functional indices.41 With ryanodine administered intracoronarily, in the current study, effects due to altered loading conditions were minimized, but the most frequently used indices of diastolic LV function (eg, tau) could not be used, because diastolic function is usually assessed for global LV function. Accordingly, we examined the pattern of relaxation selectively in the posterior wall in response to intracoronary ryanodine. The wall thinning normally observed during isovolumic relaxation of the left ventricle was replaced by either no change or a prolonged thickening in both groups of dogs after ryanodine administration. However, the changes in the slope of the posterior wall motion in the territory perfused with ryanodine were greater in control dogs than in dogs with LVH, suggesting a more potent impairment of relaxation in control dogs than in dogs with LVH. Therefore, ryanodine exerts less of a negative inotropic and less of a negative lusitropic effect on the hypertrophied myocardium of conscious dogs. Interestingly, in failing human myocardium, which also generally involves hypertrophy, there was a diminished stimulation of Ca2+ accumulation by ryanodine18; this is consistent with the physiological data presented in the current study.

A recent study in young spontaneously hypertensive rats, characterized by enhanced cardiac function at baseline, found increased responsiveness to ryanodine.42 These results, although apparently inconsistent with our results, can actually be reconciled readily. First, Mill et al42 did not measure ryanodine receptors, but if they did, downregulation would not be likely, since this is not observed in mild LVH. Secondly, the late phase of severe LVH is characterized by depressed cardiac function, as was observed in the current investigation (Figure 4). Importantly, Bers’ laboratory has shown that there are marked differences in ryanodine receptor density among different species and that the depressant effects of ryanodine correlate with the receptor density.43,44 We observed a similar relationship between ryanodine receptor density (higher in normal than in LVH hearts) and depressant effects of ryanodine (greater in normal than in LVH hearts). Because in severe LVH there was also a diminished response to ryanodine, it appears that both down-regulation of ryanodine receptors and reduced responsiveness to ryanodine presage the decrease in LV function with increasing severity of LVH.

Before concluding that the differences in LV function in response to intracoronary ryanodine were due to the down-regulation of the ryanodine receptors, it was important to
eliminate the possibility of a dilutional effect. It is unlikely that less drug was delivered to the heart because of differences in coronary blood flow. In this model of LVH, myocardial blood flow per gram of tissue is normal under baseline conditions.26,28 Furthermore, mean coronary blood flow velocity did not differ between control dogs and dogs with LVH in the current study. However, to account for this possible source of dilutional error with intracoronary drug delivery, one analysis used a comparison of a 3-fold increase in dose to the LVH dogs compared with control dogs (Table). Even under these conditions, intracoronary ryanodine exerted significantly greater effects on both systolic and diastolic regional function in control dogs (Table). Finally, in a subgroup of dogs, when norepinephrine was directly infused into the circumflex coronary artery and norepinephrine plasma levels were measured in the coronary sinus, no differences were observed between control and hypertrophied hearts, eliminating the possibility of a dilutional effect (data not shown).

One other source of error for the physiological experiments must be addressed. Although some calcium entry blockers (eg, dihydropyridine compounds) elicit marked coronary vasodilation45 and could affect drug delivery to the heart, in the present study ryanodine did not exert a major effect on coronary blood flow velocity in control dogs. Similar findings were observed in dogs with LVH, indicating a relative lack of effect of ryanodine on coronary vasoactivity. Prior to in vitro studies on the effects of ryanodine on coronary vessels also demonstrated that ryanodine affects coronary vasoactivity only modestly.5,40,45 Therefore, drug delivery was similar in both groups of dogs. Apparently, cardiac muscle is more sensitive to ryanodine than coronary vascular tissue.

Finally, it is important to recognize that the changes in transmural ryanodine receptor distribution may not be the only cause of the functional alterations noted, but rather an important but parallel event. Potentially equally as important are the total decrease in ryanodine receptors and changes in SERCA, phospholamban, and other SR proteins. More definitive proof of the causal role of altered ryanodine receptor distribution in LVH and LV failure awaits experiments with effective blockers or genetic alterations in mice, as well as documentation of changes in calcium regulatory proteins.

In summary, normally there is a transmural gradient of ryanodine receptors and message from subendocardium to subepicardium. This normal gradient is lost in LVH, as ryanodine receptors are reduced preferentially in the subendocardium in severe LVH. This is accompanied by attenuation of the action of ryanodine to depress both systolic and diastolic LV function when it is administered either intravenously or intracoronarily. In view of the correlation between ryanodine receptors and the degree of LVH, the more important correlation with the function of the hypertrophied heart, and the knowledge that the development of heart failure in this model is preceded by a decline in subendocardial LV function as well as a decrease in subendocardial coronary reserve,27,28 it is interesting to speculate that the decrease in ryanodine receptors and decreased responsiveness to ryanodine presage the decoupling from stable LVH to LV failure.

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References


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Luc Hittinger, Bijan Ghaleh, Jie Chen, John G. Edwards, Raymond K. Kudej, Mitsunori Iwase, Song-Jung Kim, Stephen F. Vatner and Dorothy E. Vatner

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