Altered Calcium Handling in Left Ventricular Pressure-Overload Hypertrophy as Detected With Aequorin in the Isolated, Perfused Ferret Heart

Lisa A. Bentivegna, LeeAnn W. Ablin, Yasuki Kihara, and James P. Morgan

The purpose of this study was to test the hypothesis that systolic and diastolic dysfunction in left ventricular pressure-overload hypertrophy is caused by abnormal intracellular calcium handling. Experiments were performed with intact, buffer-perfused, isovolumic ferret hearts (n=9 hypertrophied, n=9 control) that were loaded with the bioluminescent indicator aequorin to monitor changes in cytoplasmic calcium. In each experiment, left ventricular pressure and intracellular calcium transients were simultaneously recorded. Compared with their age-matched controls, significant hypertrophy of the left ventricle developed 4 weeks after postvalvular aortic banding; at the time the animals were killed, the left ventricular weight/body weight ratio was increased in the banded animals (5.3x10^{-3} versus 3.6x10^{-3}, p<0.001). As indicated by the diastolic pressure-volume relation, left ventricular distensibility was significantly diminished in the hypertrophied hearts. In comparison to the controls, the hypertrophied hearts demonstrated a prolonged duration of isovolumic contraction (time to 90% decline from peak: 278±5.4 versus 247±10.2 msec, p<0.05), but a marked decrease in peak systolic midwall stress (22.4±5.0 versus 38.6±5.7 g/cm², p<0.05). The increased duration of isovolumic contraction correlated with a similar prolongation of the calcium transient (time to 90% decline from peak: 245±19.5 versus 127±13.2 msec, p<0.05), indicating that the rate of sequestration and perhaps release of calcium by the sarcoplasmic reticulum is decreased in hypertrophy. In contrast, control and hypertrophied hearts had similar peak systolic calcium levels (pCa: 6.4±0.2 versus 6.6±0.1, p=NS), indicating that the diminution in peak left ventricular midwall stress developed by the hypertrophied hearts was not due to decreased availability of activator calcium. We conclude that the prolonged time course of left ventricular pressure development, but not the diminished peak isovolumic midwall stress or decreased diastolic distensibility, may be related to alterations in intracellular calcium handling. (Circulation Research 1991;69:1538–1545)

Various models of pressure-overload hypertrophy demonstrate alterations in myocardial structure and function.1–4 Impairment of contractile function, abnormalities in myocardial energetics, and alterations in calcium handling have been documented in papillary muscles and trabeculae carneae from the right ventricle.5–7 However, because these types of experiments require the use of isolated muscles with a cross-sectional area of ≤1 mm² to avoid core hypoxia, study of the left ventricle has been limited by lack of availability of suitably sized muscles.8,9 In most larger mammals, left ventricular papillary muscles are usually ≥1 mm², even in the absence of significant hypertrophy.

The role of calcium in cardiac muscle contraction of both normal and diseased hearts has been well recognized and characterized. For a concise review of this topic, refer to Dhalla et al.10 Our laboratory recently developed a technique for introducing aequorin into left ventricular myocytes of isolated, isovolumic working ferret hearts.11 With this technique, it is possible to directly study calcium handling by the left ventricle in models of disease states that cannot readily be studied with isolated muscle tech-
Table 1. Morphological Characteristics of Whole Hearts From Control and Aortic-Banded Ferrets

<table>
<thead>
<tr>
<th>Control (n=9)</th>
<th>Aortic-banded (n=9)</th>
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<tbody>
<tr>
<td>Heart weight (mg)</td>
<td>5.27±0.18</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>3.61±0.13</td>
</tr>
<tr>
<td>LV weight (g)</td>
<td>0.95±0.07</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>0.483±0.03</td>
</tr>
<tr>
<td>RV weight (g)</td>
<td>0.2</td>
</tr>
<tr>
<td>Calculated wall thickness (cm)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SEM. LV weight, weight of left ventricle; RV weight, weight of right ventricle; n, number of preparations studied.

*p<0.001, †p<0.01 vs. control (determined by Student’s t test).

FIGURE 1. Superimposed recordings of Ca²⁺ transients (upper panel) and isovolumic left ventricular pressure tracings (lower panel) in control (CONT., solid line) and hypertrophied (HYPERT., dashed line) ferret hearts. The aequorin signal is expressed as nanoamperes of anode current recorded from the photomultiplier; left ventricular pressure is expressed as millimeters of mercury.

FIGURE 2. Left ventricular (LV) end-diastolic pressure-volume curve in control (closed circles) and hypertrophied (open circles) ferret hearts. LV intraventricular balloon volumes in milliliters are plotted on the abscissa. LV isovolumic end-diastolic pressure in millimeters of mercury is plotted on the ordinate. Measurements were obtained at 30°C in 1 mM perfusate Ca²⁺. *p<0.05 vs. control.
were then intubated, and positive pressure ventilation was maintained with a respirator (Harvard Apparatus, South Natick, Mass.). A right thoracotomy was performed at the third intercostal space, and a hand (2 mm i.d.) was affixed to the aorta distal to the right subclavian artery. For 5 days after the surgical procedure, all animals were treated with subcutaneous antibiotic therapy (12,500 units penicillin G potassium, three times daily). As the animals matured for a period of 35±7 days, the degree of aortic constriction became more severe, resulting in a subacute form of pressure overload of the left ventricle.

**Experimental Procedure**

Animals were killed 4–6 weeks postbanding. After 1,000 units heparin was administered intraperitoneally, ferrets were anesthetized by mask induction with chloroform. Hearts were quickly excised and mounted on a cannula inserted into the ascending aorta. Retrograde perfusion of the coronary arteries was established via a constant flow pump with the coronary perfusion pressure initially set at 80 mm Hg as monitored by a Statham P23Db transducer. Hearts were perfused with a modified Krebs-Henseleit solution composed of (mM) NaCl 118, KCl 4.7, KH₂PO₄ 1.2, CaCl₂ 1.0, MgCl₂ 1.2, NaHCO₃ 23, dextrose 5.5, and lactate 1.0 and saturated with a 95% O₂–5% CO₂ gas mixture to a pH of 7.4±0.2.

Isovolumic left ventricular pressure was recorded via an intraventricular balloon catheter inserted into the left ventricle through the mitral valve and connected to another Statham P23Db transducer. Coronary sinus effluent was collected through a pulmonary artery catheter, while thebesian venous outflow was emptied from the left ventricle via a drain from the ventricle placed in parallel with the balloon catheter. Ventricular pacing was established with a platinum pacing wire inserted through a right atrial incision. Heart rate was maintained constant at 100 beats/min. A bipolar electrocardiogram was obtained from a positive electrode sutured on the exterior of the left ventricle between the left anterior descending and left circumflex branches of the left main coronary artery, and the negative lead was sutured to the right ventricle near the pulmonary outflow tract.

After surgical manipulation of the preparation was complete, hearts were allowed to equilibrate for 30 minutes before preparation for aequorin loading. After this stabilization period, a localized region of the left ventricle was macroinjected with aequorin as previously described. In brief, hearts were cooled to 16°C and perfused with zero calcium solution. Within 3 minutes, hearts became asystolic and coronary

**Figure 3.** Effect of increasing perfusate calcium on aequorin light signal and left ventricular pressure in control (upper tracings) and hypertrophied (lower tracings) ferret hearts. Aequorin light signals are shown above and left ventricular pressures are below in both sets of tracings. Note the corresponding increase in light and pressure as calcium increases in the control. In contrast, in the hypertrophied hearts, pressure reaches a plateau at [Ca²⁺]₀=2 mM, despite a continued increase in peak light at higher [Ca²⁺]₀.
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resistance fell. After 15 minutes of zero calcium perfusion, coronary perfusion was reduced to achieve a perfusion pressure of 20–25 mm Hg in preparation for aequorin loading. Aequorin solution was then injected with a low-resistance glass micropipette into the interstitium of the epicardium at the apex of the left ventricle.

Hearts were enclosed in a light-tight box designed for aequorin studies by Blinks and modified for the Langendorff-perfused heart as previously described by Kihara et al. In brief, this system consists of an ellipsoidal mirror with the aequorin-loaded region of the heart positioned at one focal point and the photocathode of a photomultiplier tube (9635QA, Thorn-EMI, Gencom, Inc., Fairfield, N.J.) at the other. This apparatus is used to maximize collection of light emitted from the aequorin-loaded site.

After aequorin loading, calcium was gradually re-introduced to the perfusate, and coronary flow rate and temperature were restored to preloading conditions while the aequorin light signal reached a steady state. At this point, pressure-volume relations were obtained through systematically varying preload conditions by increasing the intraventricular balloon volume. Aequorin light signals, left ventricular isovolumic pressures, perfusion pressures, and electrocardiograms were recorded simultaneously on strip-chart recording paper and magnetic tape.

In preparation for determining the concentration-response relation to calcium, the perfusate was replaced with a zero phosphate solution to avoid precipitation of calcium salts. Increasing concentrations

![Figure 4](image)

**Figure 4.** Concentration–response curve for Ca²⁺ in control (closed circles) and hypertrophied (open circles) ferret hearts. Left ventricular pressures are normalized for heart weight and geometry and expressed as peak systolic midwall stress in g/cm². See text for details. *p<0.05 vs. control.

![Figure 5](image)

**Figure 5.** Concentration–response curve to Ca²⁺ in aequorin-loaded whole hearts from control (closed circles) and hypertrophied (open circles) ferret hearts. Panel A: Isovolumic pressure response expressed as percent of maximal pressure. Panel B: Peak light response represented as percent of maximal light. *p<0.05 vs. hypertrophied hearts.
of calcium were added to the perfusate to obtain a dose–response relation.

Quantitation of calcium was performed by the method of fractional luminescence. At the conclusion of the experiment, six hearts from the control and five from the banded groups were perfused with 8 mM calcium and 5% Triton X-100 to lyse the cells and expose all of the remaining aequorin to calcium. This resulted in an instantaneous burst of light that declined quickly to baseline. The area under the resultant curve was then integrated to obtain a value for \( L_{\text{max}} \), the total amount of light emitted from the aequorin in the heart. These results were then compared with in vitro calibration curves to obtain \(-\log \) values. Left ventricular wall thickness and peak systolic midwall stresses were calculated at various points in each experiment by using the Young-La Place thin wall method as reported by Mirsky. Data were reported as the mean±SEM of the control versus banded groups.

Results

Nine aortic-banded and nine age-matched control male ferrets were studied in this series. At the time the animals were killed, control ferrets (mean body weight of 1.06±0.3 kg and mean age of 106±4 days) were not significantly different from banded ferrets (mean weight of 0.90±0.1 kg and mean age of 107±3 days). Table 2 lists the mean morphological characteristics of hearts from the control and banded groups. The ratios of heart weight to body weight and left ventricular weight to body weight were significantly higher in the aortic-banded group, indicating that left ventricular hypertrophy was present in the banded animals. No difference was found in the right ventricular weight/body weight ratio between these groups, indicating that compensatory right ventricular hypertrophy was not present in the banded group. At a mean end-diastolic pressure of 11.8±1.14 mm Hg in the control group and 15.2±1.10 mm Hg in the hypertrophied group, the calculated wall thickness was significantly greater in the hypertrophied group (\( p<0.05 \)).

Superimposed aequorin signals and left ventricular pressure tracings recorded from representative control and hypertrophied hearts are shown in Figure 1. In both hearts, note the characteristic rapid rise of the aequorin light signal that precedes the corresponding mechanical event. In the hypertrophied preparation both the \( \text{Ca}^{2+} \), transient and isovolumic relaxation are prolonged, as indicated by the arrows.

Figure 2 demonstrates the relation between left ventricular balloon volume and end-diastolic pressure. Diastolic pressures were significantly higher in the hypertrophied hearts at balloon volumes greater than 0.1 ml (\( p<0.05 \)).

Figure 3 demonstrates the effect of increasing perfusate calcium concentration on the aequorin light signal and left ventricular function. Although no differences in peak systolic pressures were noted at any calcium concentration, when normalized for left ventricular geometry and expressed as peak systolic midwall stress, contractile function was depressed in

**Table 2. Fractional Luminescence Responses**

<table>
<thead>
<tr>
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<th>Resting</th>
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<th>Peak</th>
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<tr>
<td></td>
<td>( -\log (L/L_{\text{max}}) )</td>
<td>( \text{pCa} )</td>
<td>( -\log (L/L_{\text{max}}) )</td>
</tr>
<tr>
<td>Control (( n=6 ))</td>
<td>5.42±0.10</td>
<td>6.6±0.06</td>
<td>4.68±0.13</td>
</tr>
<tr>
<td>Hypertrophy (( n=5 ))</td>
<td>5.07±0.31</td>
<td>6.4±0.19</td>
<td>4.28±0.43</td>
</tr>
</tbody>
</table>

Values are expressed as the negative log of the fractional luminescence \( (L/L_{\text{max}} \) see text for details) and as \( \text{pCa} \), the negative log of \( [\text{Ca}^{2+}] \). All data for resting and peak \( \text{Ca}^{2+} \) were determined at 1 mM perfusate \( [\text{Ca}^{2+}] \) at 30°C. No significant difference was found between control and hypertrophied hearts.
the banded group ($p<0.05$) at 2 and 8 mM perfusate calcium, as indicated in Figure 4.

The percent of maximal developed pressure and light were compared at increasing calcium concentrations (Figure 5). At lower calcium concentrations, the hypertrophied hearts appeared to be more maximally activated because the dose–response relation was positioned to the left of the controls. At 2 mM extracellular calcium and above, the curves were virtually superimposable at each calcium concentration studied.

To test the $Ca^{2+}$ responsiveness of control and hypertrophied hearts, the calcium concentration–response relation was determined. Time to peak and time to 90% decline of the pressure and light tracings were plotted versus perfusate calcium concentration as shown in Figure 6. The time to peak pressure was significantly longer in the hypertrophied hearts in 8 mM calcium, whereas time to 90% decline from peak pressure was prolonged at 1, 2, and 4 mM extracellular calcium. The times to peak of the calcium transients were similar in both groups, whereas the hypertrophied hearts demonstrated a prolonged time to 90% decline from peak light at all $[Ca^{2+}]_0$ tested.

The values of peak systolic and end-diastolic $Ca^{2+}$ values in 1 mM $[Ca^{2+}]_0$ are shown in Table 2. No differences in $Ca^{2+}$ levels were detected between these two groups.

**Discussion**

One of the most important findings of this study is that the prolongation of the contractile state of the hypertrophied left ventricle, as seen in the upper right-hand panel of Figure 6, correlates with prolongation of the intracellular calcium transient (lower right-hand panel of Figure 6). Our results confirm and extend those of isolated muscle studies that demonstrate temporal differences in the contractile function and calcium handling of hypertrophied myocardium. Previous aequorin studies have documented prolongation of twitch duration and the corresponding $Ca^{2+}$ transient in papillary muscles and trabeculae carneae isolated from various models of hypertrophy, including right ventricular pressure.

![Figure 7](image-url) **Figure 7.** Continuous recording of left ventricular isovolumic pressure (expressed in millimeters of mercury) and aequorin light signal (representative of intracellular calcium, expressed in nanoamperes of anodal current) from a control ferret heart. This heart was paced at 1.5 cycles/sec. Note the consistency of the amplitude of the aequorin signal with respect to the left ventricular pressure.

![Figure 8](image-url) **Figure 8.** Continuous recording of left ventricular isovolumic pressure (expressed in millimeters of mercury) and aequorin light signal (expressed in nanoamperes of anodal current) in a heart isolated from an aortic-banded ferret. Note the beat-to-beat variability of the aequorin transient correlating with ventricular ectopy.
overload in the ferret \(^7\) and end-stage dilated and hypertrophic cardiomyopathy in humans. \(^{16}\) However, this report is the first documentation of these abnormalities in calcium handling in left ventricular muscle of the intact heart. Similar findings have been shown in isolated human ventricular myocytes with the fluorescent Ca\(^{2+}\) indicator fura-2. \(^{17}\) Consistent with these findings, the velocity of shortening of isolated hypertrophied feline myocytes has been shown to be slow compared with nonhypertrophied controls, similar to findings in isolated muscle studies. \(^{18,19}\) As discussed above, the aequorin-loaded whole-heart technique allows the study of left ventricular calcium regulation and contractile function in the presence of hypertrophy and disease states without the limitations posed by isolated muscle techniques. An advantage of this technique is that it allows investigators to examine beat-to-beat variability of intracellular calcium with each corresponding cardiac cycle. It was noted in this series of experiments that the aortic-banded animals exhibited a greater frequency of ectopy and required an increased stimulation voltage to achieve pacing (Figures 7 and 8).

As shown in Figure 1 and the lower right-hand panel of Figure 6, the aortic-banded pressure-overload model of left ventricular hypertrophy exhibits abnormalities in the time course of calcium handling. Prolongation of the aequorin light signal appears to predominantly reflect the inability of the sarcoplasmic reticulum to resequester Ca\(^{2+}\) \(^{12}\) during diastole. \(^{20-23}\) The prolongation of the calcium transient, observed in the present study, corresponds closely with a decreased rate of decline of left ventricular pressure, indicating impaired diastolic relaxation (Figures 1 and 6). In vivo, this diastolic dysfunction might result in impaired left ventricular filling, particularly at the usual ferret heart rate of 250 beats/min. \(^{24}\) It should be noted, however, that overt clinical signs of failure were not observed, and compensatory right ventricular hypertrophy was not present in any of the banded animals.

Figure 4 shows that calculated peak systolic midwall stress was decreased in the hypertrophied hearts and was not corrected to control values by the elevation of calcium in the perfusate. These results indicate that the lesser ability to generate wall stress is not due to decreased availability of activator Ca\(^{2+}\). Similar wall stress findings have been shown by Pfeffer et al. \(^{25}\) in the genetically induced spontaneously hypertensive rat with long-standing hypertrophy.

To directly test the finding that the decrease in peak midwall stress seen in the hypertrophied animals was not due to a decreased availability of Ca\(^{2+}\), the amplitudes of the aequorin transients from control and hypertrophied hearts were compared. Peak values of \(L/L_{\text{max}}\) were similar between the control and hypertrophied groups as seen in Table 2, directly indicating that the availability of calcium for activation of the myofilaments was not diminished and therefore cannot account for the contractile abnormalities in hypertrophied myocardium.

Values obtained in this study for resting calcium levels as measured by \(L/L_{\text{max}}\) were similar for the control and hypertrophied groups, although they are slightly higher than the resting values reported previously by our group in the whole-heart model. \(^{11}\) This may be due to the fact that before cell lysis with Triton X-100, these hearts were subjected to several hours of physiological and pharmacological interventions that may have resulted in cellular Ca\(^{2+}\) loading or myocyte damage.

Left ventricular end-diastolic pressures were compared by plotting the pressure–volume relation as shown in Figure 2. Initially, no difference between control and hypertrophied animals was evident, although with increasing ventricular balloon volumes, the curve for the hypertrophied hearts was shifted to the left compared with the controls. This difference in diastolic properties indicates decreased distensibility of the hypertrophied left ventricle in the late diastolic phase. Because no difference was found between diastolic calcium levels in the control and hypertrophied ventricles, it appears that this decreased distensibility is not due to altered calcium handling. In addition to increased muscle mass and abnormal ventricular geometry, hypertrophy has been shown to result in additional structural and functional changes that may affect distensibility, including an increase in collagen content, \(^{26}\) an increase in capillary luminal volume without a corresponding increase in capillary length, \(^{27}\) and a decreased mitochondrial/myofibrillar ratio. \(^{28}\)

To test the possibility that decreased sensitivity of the myofilaments to calcium might account for the abnormalities of contractile function in the hypertrophied myocardium, the percent change from maximal pressure and the peak light signal were determined. No change was found in either percent of maximum light or pressure at or above physiological calcium levels, indicating that the calcium sensitivity of the myofilaments is not significantly altered in the hypertrophied myocardium.

Many studies of pressure-overload hypertrophy have documented abnormal calcium handling as an important contributor to the altered contractile function that characterizes hypertrophied myocardium. These abnormalities have been documented in cellular components including the sarclemma, sarcoplasmic reticulum, and mitochondria. \(^{29}\) The importance of the present study with respect to the existing literature is the ability to measure these changes in calcium handling simultaneously with global cardiac function, without the restrictions imposed on isolated tissue techniques. In vitro, left ventricular studies have been restricted because of muscle size, with the danger of inducing core hypoxia in muscles with a diameter of greater than 1 mm. The technique used in the present study provides results comparable to those obtained with the isolated muscle and single cell technique but, in addition, allows the examination of pathophysiological states of the left ventricle that are not able to be studied using the previously
mentioned techniques. This study documents that the changes in systolic and diastolic properties of the hypertrophied left ventricle are due, at least in part, to abnormal calcium handling.

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Key Words • calcium • aequorin • hypertrophy • diastolic dysfunction • pressure overload
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