Intracellular Calcium Transients in Myocardium From Spontaneously Hypertensive Rats During the Transition to Heart Failure

Oscar H.L. Bing, Wesley W. Brooks, Chester H. Conrad, Subha Sen, Cynthia L. Perreault, and James P. Morgan

To investigate the mechanism of impaired myocardial function after long-term pressure overload, we studied cardiac muscle mechanical contraction and intracellular calcium transients using the bioluminescent indicator aequorin. Left ventricular papillary muscle preparations were examined from three groups of rats: 1) aging spontaneously hypertensive rats (SHR) with clinical and pathological evidence suggesting heart failure (SHR-F group), 2) age-matched SHRs with no evidence of heart failure (SHR-NF group), and 3) age-matched normotensive Wistar-Kyoto rats (WKY group). Isometric force development was depressed in both SHR groups relative to the WKY group. Resting [Ca\(^{2+}\)] in was lower in the SHR-F group, and the time to peak [Ca\(^{2+}\)] in was prolonged in this group. The relative increases in peak [Ca\(^{2+}\)] with the inotropic interventions of increased [Ca\(^{2+}\)], and the addition of isoproterenol were similar among groups. Although inotropy increased in all groups with increased [Ca\(^{2+}\)], after isoproterenol, inotropy increased only in the WKY group. Thus, in SHR myocardium, [Ca\(^{2+}\)], increased after isoproterenol, but inotropy failed to increase. Myosin isoforms were shifted toward the V\(_3\) isoform in both SHR groups; the V\(_3\) isoform was virtually 100% in papillary muscles from the SHR-F group. These changes may reflect events directly contributing to the development of heart failure or represent adaptive changes to chronic pressure overload and heart failure. (Circulation Research 1991;68:1390–1400)

Although hypertrophy is a functionally important compensatory response of the myocardium to an increased load, stable hypertrophy may progress to a decompensated state, with cardiac pump dysfunction and myocardial depression. Altered intracellular calcium handling has been described in a number of pathophysiological conditions involving the myocardium, and it has been suggested that calcium overload is a factor that may lead to myocardial necrosis. The spontaneously hypertensive rat (SHR) is a well-established model of genetic hypertension, in which cardiac pump function is preserved at 1 year of age. At 18 months and older, however, SHR demonstrate a reduction in peak stroke volume and cardiac index. Isolated papillary muscle performance is normal or enhanced in SHR up to 18 months of age. At 18–24 months, SHR with left atrial thrombi, pleural and pericardial effusions, and right ventricular hypertrophy demonstrate clear depression of papillary muscle function, as well as increased stiffness, which correlates with histological and biochemical evidence of fibrosis. These data are consistent with a transition in the functional status of the myocardium, resulting in a decline of muscle and pump function. It was the purpose of this study to investigate intracellular calcium levels in a model of long-standing left ventricular hypertrophy in which animals are observed to develop clinical and pathological findings consistent with heart failure.
Materials and Methods

Animal Model

Male SHR and normotensive Wistar-Kyoto (WKY) control rats, 6–9 months of age, were purchased as retired breeders from Taconic, Germantown, N.Y., and boarded in the animal facility at the Boston VA Medical Center until the time of study (at 18–24 months of age). We have previously found that a number of SHR demonstrate evidence of heart failure beginning at the age of 18 months. Therefore, after the age of 18 months, the rats were observed on a daily basis. When SHR were observed to develop tachypnea and labored respiration, they were studied within 1–2 weeks. No WKY rats developed respiratory difficulties. A few SHR with respiratory findings died before they could be studied and, at autopsy, had the pathological findings consistent with heart failure, as described below. Left ventricular papillary muscle preparations were examined from three groups of rats: 1) aging SHR with evidence of heart failure (SHR-F), 2) age-matched SHR without evidence of heart failure (SHR-NF), and 3) age-matched normotensive WKY rats. Tail-cuff blood pressures were obtained in all rats on the day before the study.

Isolated Muscle Performance

At the time of study, the rats were decapitated. Their hearts were quickly removed and placed in oxygenated bicarbonate-buffered physiological salt solution at 30°C. A left ventricular papillary muscle was carefully dissected free. The base of the muscle was fixed to a muscle holder, and the tendinous end was connected to a strain-gauge tension transducer. The preparation was mounted in a chamber containing a physiological salt solution of the following composition (mM): NaCl 120, KCl 5.9, NaHCO3 25, NaH2PO4 1.2, MgCl2 1.2, CaCl2 1.0, and dextrose 11.5. The solution was bubbled with 95% O2–5% CO2 and equilibrated to pH 7.4 at 30°C. To prevent precipitation of calcium, phosphate was deleted during experiments in which [Ca2+]o was varied. The muscle preparation was stimulated via a punctate platinum electrode located at the base of the muscle, at a rate of 0.33 Hz, using square-wave pulses 5 msec in duration. The voltage was set to 10% above threshold. After a 30-minute equilibration period, the muscle preparation was carefully lengthened to the apex of the active tension–length relation. After a 5-minute period of stable isometric contractions at this length, the following isometric contraction parameters were recorded: resting tension (in grams per square millimeter), active tension (defined as peak isometric tension minus resting tension, in grams per square millimeter), maximum rate of isometric tension development (dT/dt, in grams per square millimeter per second), time to peak tension (TPT, in milliseconds), and time from peak isometric tension to 50% peak isometric tension (RT1/2, in milliseconds). At the conclusion of the experiment, the muscles were blotted and weighed. Cross-sectional area was determined from muscle weight and length by assuming a uniform cross section and a specific gravity of 1.05. After removal of the papillary muscle for study, the cardiac chambers (atria and right and left ventricles) were weighed. Cardiac chamber weights, normalized by body weight, were used as indexes of hypertrophy.

Aequorin Signal Measurements

Aequorin was loaded into the muscle preparations by the macroinjection technique. In brief, muscle bath temperature was lowered to 20°C. The solution bathing the muscle was changed to one of similar composition, but it contained no added calcium; thus, the calcium concentration was in the range of 10−7–10−6 M. Stimulation was carried out until tension fell to <50% of baseline values in 1.0 mM [Ca2+]o, and then it was discontinued. After a 5-minute quiescent period, the preparation was raised from the bath, and a short-shank low-resistance glass micropipette containing ∼1–2 μl aequorin solution (1 mg/ml, purchased from the laboratory of Dr. J.R. Blinks, Mayo Clinic, Rochester, Minn.) was carefully introduced through the epimysium of the muscle. The aequorin-loading process required ∼30 seconds. The muscle was then lowered into the bath, and calcium was reintroduced at 20-minute intervals to the following concentrations (mM): 0.001, 0.01, 0.1, and 1.0. After an additional 20-minute period, preparations were gradually rewarmed to 30°C and then stimulated to contract. Muscles were allowed to equilibrate for a 90–120-minute period until a steady state was achieved. Preparations in which active tension did not recover to at least 65% of before-loading values were discarded.

A light-collecting apparatus designed by Blinks was used to simultaneously measure the aequorin luminescence signal (light signal) and isometric tension. The output of the photomultiplier (model 9635QA, Thorn-EMI Electron Tubes, Fairfield, N.J.) was converted to a voltage signal by a preamplifier with a time constant of 10 msec. Both light and tension signals were recorded on magnetic tape. To improve the signal-to-noise ratio, between 10 and 200 signals were averaged while the muscle contracted in the steady state. Parameters derived from the light signal included the amplitude of the transient, time to peak light (TPL, in milliseconds), and time from peak to 50% fall in peak light (LT1/2, in milliseconds).

To compare aequorin signals from the different groups of rats, the method of fractional luminescence was used. The light intensity under conditions in which all of the aequorin was exposed to a saturating concentration of calcium (I100) was determined by lysing the preparation with a 5% solution of Triton X-100 (Fisher Scientific Co., Pittsburgh, Pa.) in phosphate-free physiological salt solution containing 50 mM Ca2+ at 30°C. After subtraction of the dark current, total luminescence was determined by integrating the area encompassed by the aequorin signal after Triton treatment. Values were then corrected
for the rate constant of aequorin consumption (2.11/ sec), which was determined in the presence of saturating calcium at 30°C after preincubation of aequorin with 1 mM Mg²⁺. Fractional luminescence (L/Lmax) was determined from the muscle preparation at rest (resting light) and at maximum amplitude of the aequorin signal (peak light). For conversion of L/Lmax to [Ca²⁺], a calibration curve was determined from in vitro data. The relation between [Ca²⁺] and L/Lmax was then fitted to the formula:

\[ \frac{L}{L_{\text{max}}} = \left( 1 + K_h \cdot [Ca^{2+}] \right) / \left( 1 + K_s \cdot [Ca^{2+}] \right) \]

where the constants \( K_h \) and \( K_s \) were determined by using a nonlinear regression analysis with iteration (\( K_h = 4.50 \times 10^{-6} \text{M} \) and \( K_s = 130.0 \)). This formula was used for the calculation of [Ca²⁺], from fractional luminescence data. Based on data from Kihara et al, we estimate that the minimum [Ca²⁺], we could reliably detect is 0.1 \( \mu \text{M} \).

Myosin Isozyme Studies

Papillary muscle preparations from the WKY, SHR-NF, and SHR-F groups were flash-frozen in liquid nitrogen and stored in liquid nitrogen for later myosin isozyme analysis. At the time of analysis, 5–10 mg tissue was minced with small sharp scissors and washed in phosphate-buffered saline (40 mM NaCl and 3 mM Na₂HPO₄, pH 7.0 at 2°C). The tissue was then homogenized in 7 ml saline buffer with the use of a ground glass tissue grinder (Kontes, Morton Grove, Ill.) for a period of 90 seconds (three 30-second homogenizations). The homogenate was then centrifuged at 3,000 rpm for 10 minutes at 2°C (model RC5B, Sorvall, Wilmington, Del.). The supernatant was discarded, and the pellet was washed with 7 ml phosphate-buffered saline and centrifuged at 3,000 rpm for 10 minutes. The supernatant was again discarded. The pellet was resuspended in 3 ml extracting solution containing 100 mM Na₃P₂O₇, 5 mM EGTA, and 5 mM dithiothreitol, pH 6.8. The homogenate was shaken in an ice bath in a walk-in cold room (4°C) for 60 minutes and then centrifuged at 20,000 rpm in a Sorvall model RC5B centrifuge using an SM-24 rotor for 2 hours at 2°C. The supernatant was collected and mixed with an equal volume of ice-cold glycerol and stored at -20°C.

Polyacrylamide gel electrophoresis using 4% gels was done according to the method of Hoh et al. Gels consisted of an acrylamide stock solution (200 g/l acrylamide and 6.2 g/l \( N, N' \)-methylene-bis-acrylamide), 3.1 ml pyrophosphate stock solution (27 mM Na₃P₂O₇; and 13.4% vol/vol glycerol, pH 8.8), and 22 \( \mu \text{L} \) \( N, N', N'', N''' \)-tetramethylethylenediamine; polymerization was initiated by adding 35 \( \mu \text{L} \) freshly prepared ammonium persulfate solution (125 g/l).

Electrophoresis was carried out in a Pharmacia electrophoresis apparatus (model GE2/4, Pharmacia LKB Biotechnology Inc., Piscataway, N.J.). The electrophoresis buffer contained 20 mM Na₃P₂O₇ and 10% (vol/vol) glycerol, pH 8.8 at 2°C. The buffer was recirculated during the run to neutralize the products of electrolysis formed during the run. A 60-minute prerun was carried out before myosin was applied to the gel, using a current of 2 mA/gel. Myosin was loaded directly on top of the gel. The current of 2 mA/gel was maintained for 5 hours, after which the gels were run under constant voltage supplied by a constant voltage power supply (model 3-1500, Buchler Instruments, Fort Lee, N.J.).

The gels were stained for protein in 10 ml Coomassie brilliant blue R solution (0.3 g/l) for 2 hours at room temperature. Gels were then destained in a 7% (vol/vol) acetic acid and 30% (vol/vol) methanol solution in a gel electrophoresis diffusion destainer (model 171A, Bio-Rad, Richmond, Calif.). Myosin isozymes were quantified by densitometric scanning of the gels with the use of a Quick Scan R&D densitometer (Helena Laboratories, Beaumont, Tex.).

Protocol

Baseline isometric contraction parameters were recorded from all muscle preparations after muscle equilibration at the apex of the active tension-length relation. All muscle preparations were then loaded with aequorin as described. Concentration–response relations to [Ca²⁺]i (0.625–5.0 mM) and isoproterenol (10⁻⁸–10⁻⁶ M in 1.0 mM [Ca²⁺]i) were studied in SHR-F (n=6), SHR-NF (n=7), and WKY (n=7) rats. Three other groups of rats, SHR-F (n=4), SHR-NF (n=8), and WKY (n=6), were used for fractional luminescence determinations as described above. These determinations were not carried out in muscles exposed to isoproterenol because these preparations did not return to baseline conditions (either mechanical activity or light).

Statistical Analysis

Data from the SHR-F, SHR-NF, and WKY groups were compared using one-way analysis of variance with replication. A two-way analysis of variance was used to examine group and treatment effects. The Newman-Keuls multiple-sample comparison test was used to localize differences where appropriate. Data are expressed as mean±SEM.

Results

Studies were conducted with 23 SHR and 22 WKY rats, 18–24 months of age. Ten SHR had findings consistent with heart failure (SHR-F group), including tachypnea (n=9), pleural and/or pericardial effusions (n=8), left atrial thrombi (n=8), and right ventricular hypertrophy (as defined by right ventricular weight/body weight ratio >0.8 mg/g, n=10). Visible fibrosis was present in all hearts in the SHR-F group and in many of the SHR-NF group. None of the WKY rats demonstrated any of these findings. Data on animal blood pressure, body weight, cardiac chamber weight, and chamber weights normalized for body weight are presented in Table 1. Tail-cuff blood pressure was elevated in both SHR groups relative to the WKY group (p<0.01) but was some-
TABLE 1. Chamber Weight, Body Weight, and Blood Pressure Data

<table>
<thead>
<tr>
<th></th>
<th>LV wt (g)</th>
<th>RV wt (g)</th>
<th>Atrial wt (g)</th>
<th>Body wt (g)</th>
<th>LV wt/body wt (mg/g)</th>
<th>RV wt/body wt (mg/g)</th>
<th>Atrial wt/body wt wt (mg/g)</th>
<th>BP (mm Hg)</th>
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<tr>
<td>WKY (n=22)</td>
<td>1.23±0.02</td>
<td>0.29±0.01</td>
<td>0.15±0.01</td>
<td>690±16</td>
<td>1.80±0.04</td>
<td>0.42±0.01</td>
<td>0.22±0.01</td>
<td>116±1</td>
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<tr>
<td>SHR-NF (n=13)</td>
<td>1.38±0.06*</td>
<td>0.20±0.01*</td>
<td>0.16±0.01</td>
<td>394±14*</td>
<td>3.45±0.09*</td>
<td>0.52±0.02*</td>
<td>0.41±0.02*</td>
<td>203±6*</td>
</tr>
<tr>
<td>SHR-F (n=10)</td>
<td>1.58±0.03†</td>
<td>0.43±0.02†</td>
<td>0.32±0.02†</td>
<td>418±7*</td>
<td>3.80±0.07†</td>
<td>1.03±0.04†</td>
<td>0.78±0.06†</td>
<td>185±6*†</td>
</tr>
</tbody>
</table>

Values are mean±SEM. LV, left ventricular; RV, right ventricular; BP, blood pressure; WKY, normotensive Wistar-Kyoto rats; SHR-NF, spontaneously hypertensive rats without heart failure; SHR-F, spontaneously hypertensive rats with heart failure.

*p<0.01 vs. corresponding value for WKY; †p<0.01 vs. corresponding value for SHR-NF.

what lower in the SHR-F group as compared with the SHR-NF group (p<0.01). Both SHR groups demonstrated left ventricular hypertrophy as manifested by an increase in the left ventricular weight/body weight ratio; this ratio was slightly greater in the SHR-F group than in the SHR-NF group. Right ventricular hypertrophy (increased right ventricular weight/body weight ratio) was seen in all rats from the SHR-F group and in no rats from the other two groups.

Isometric contraction parameters from papillary muscle preparations at the apex of the length–tension relation before aequorin loading are shown in Figure 1. There was no significant difference in muscle cross-sectional area among the groups (WKY, 1.25±0.11 mm²; SHR-NF, 1.49±0.11 mm²; SHR-F, 1.45±0.13 mm²). Active tension was significantly depressed in both SHR groups relative to the WKY group (SHR-NF, p<0.05; SHR-F, p<0.01), but there was no statistically significant difference between the SHR-F and SHR-NF groups. The depression in active tension was associated with a parallel reduction in dT/dt in both SHR groups as compared with the WKY group; dT/dt was reduced in the SHR-F group as compared with the SHR-NF group (p<0.01). No differences in TPT among groups were seen. RTT/2 was abbreviated in the SHR-NF group as compared with the WKY group (p<0.01) and further abbreviated in the SHR-F group (p<0.05 versus SHR-NF).

To examine the effects of our aequorin-loading procedure on muscle performance, we compared the pre-loading and post-loading values for the amplitude and duration of the twitch. Post-loading active tension in WKY preparations was lower than pre-loading values (3.10±0.31 g/mm² pre-loading versus 2.43±0.31 g/mm² post-loading, p<0.01); in contrast, pre-loading and post-loading values of active tension were not significantly changed in the SHR groups. The fall in active tension in the WKY group did not appear to be due to a depression of inotropy (as no significant decline in dT/dt was seen in any of the groups) but was associated with a significant abbreviation of TPT and RTT/2 (p<0.01 compared with pre-aequorin-loading data). TPT was significantly shorter in the WKY group relative to both SHR groups after loading (p<0.01), whereas RTT/2 was not different among groups. Thus, the time course of

FIGURE 1. Bar graphs showing baseline isometric contraction parameters in three rat groups: normotensive Wistar-Kyoto rats (WKY), spontaneously hypertensive rats without heart failure (SHR-NF), and spontaneously hypertensive rats with heart failure (SHR-F). Panel a: Active tension (AT). Panel b: Peak rate of tension development [(+dT/dt)/dT]. Panel c: TPT. Panel d: Time to peak tension (TPT). Data are mean±SEM. *p<0.05 and **p<0.01.
mechanical activity was abbreviated in the WKY group after the aequorin-loading procedure, whereas significantly less change was seen in the SHR groups. The extent to which these changes in the WKY group are due to the aequorin-loading procedure per se is unclear, since we have previously observed differences in the performance of WKY and SHR muscles that were time dependent. It is interesting that the apparently stronger WKY preparations are affected to a greater degree than SHR preparations, which developed considerably less force under basal conditions. Changes in peak force did not occur in preparations from either SHR group after the loading procedure.

Records with sufficient resolution to provide accurate data to determine temporal components of the aequorin transient were obtained in six SHR-F, six SHR-NF, and five WKY preparations. TPL (Figure 2a) was prolonged in the SHR-F group relative to the WKY group under basal conditions (p<0.05). No differences in L1/2 was seen among groups (Figure 2b).

When bath calcium was increased from 0.625 to 5.0 mM, peak isometric force increased in all groups (WKY, from 1.68±0.80 to 2.40±0.73 g/mm²; SHR-NF, from 1.23±0.42 to 1.94±0.71 g/mm²; SHR-F, from 1.0±0.61 to 1.81±1.02 g/mm²; all p<0.01). Adding isoproterenol at a concentration of 10⁻⁶ M in the presence of 1.0 mM [Ca²⁺], did not change peak force in the WKY group (from 2.23±0.99 to 2.18±0.62 g/mm²). Peak force declined significantly in the SHR-NF group (from 1.67±0.57 to 1.16±0.42 g/mm²; p<0.01); an apparent decrease in the SHR-F group did not reach statistical significance (from 1.4±1.02 to 1.05±0.67 g/mm²). Peak force declined, to a relatively stable plateau, within several minutes after addition of isoproterenol. The decline in peak force was not preceded by a transient increase in isometric tension (see Figure 3). Examples of the effects of increased bath calcium concentration (5.0 mM) and isoproterenol (10⁻⁶ M in 1.0 mM [Ca²⁺]) on calcium transients and mechanical activity in the three groups of muscles are presented in Figure 4.

Active tension reflects not only the rate of activation but also the duration of activation, both of which are altered by β-adrenergic stimulation. Therefore, dT/dt was used as an index of inotropy. The inotro-
[Ca$^{2+}$], was increased to 5.0 mM after addition of $10^{-6}$ M isoproterenol, a further increase in the calcium transient was noted (Figure 6b). In one experiment carried out in a rat from the SHR-NF group, inotropy in response to isoproterenol was assessed in the presence of 0.625 mM [Ca$^{2+}$]o. Findings were similar to those seen with 1.0 mM [Ca$^{2+}$]o, that is, as isoproterenol was increased from $10^{-6}$ to $10^{-5}$ M, peak force fell, inotropy failed to increase, and [Ca$^{2+}$]o increased (in this study peak tension fell 27% and peak [Ca$^{2+}$]o increased $>100\%$). Thus, an increase in peak [Ca$^{2+}$]o was seen in both SHR groups despite the absence of evidence of inotropy as manifested by the lack of an increase in dT/dt (Figure 5b).

The relation between inotropy and peak light in the presence of 5.0 mM [Ca$^{2+}$]o and isoproterenol

\[ \text{FIGURE 4.} \quad \text{Calcium transient and isometric tension recordings from left ventricular papillary muscle preparations from three rat groups: normotensive Wistar-Kyoto rats (WKY), spontaneously hypertensive rats without heart failure (SHR-NF), and spontaneously hypertensive rats with heart failure (SHR-F) (mean of 100, 50, and 10 recordings, respectively). Panel a: 0.625 and 5.0 mM [Ca$^{2+}$]o. Panel b: Control (C, 1.0 mM [Ca$^{2+}$]o) and 10$^{-6}$ M isoproterenol (ISO). Vertical calibration bars are 1.0 nA (light) and 1.0 g/mm² (tension). Note the increase in both the calcium transient and active tension with the change from 0.625 to 5.0 mM [Ca$^{2+}$]o in the WKY and both SHR groups. With ISO, the calcium transient increased in all groups, but maximum rate of isometric tension development failed to increase in the SHR groups (active tension fell, in fact, due to the reduction in the duration of tension development).} \]

\[ \text{FIGURE 5.} \quad \text{Bar graphs showing inotropic response to calcium and isoproterenol in three rat groups: normotensive Wistar-Kyoto rats (WKY), spontaneously hypertensive rats without heart failure (SHR-NF), and spontaneously hypertensive rats with heart failure (SHR-F). Panel a: Maximum rate of isometric tension development (dT/dt; control is 1.25 mM [Ca$^{2+}$]o) for 0.625, 1.25, 2.5, and 5.0 mM [Ca$^{2+}$]o, *p<0.05 and **p<0.01 vs. 1.25 mM [Ca$^{2+}$]o. Panel b: dT/dt in the baseline state (no isoproterenol), with $10^{-6}$, $10^{-7}$, and $10^{-8}$ M isoproterenol and with $10^{-6}$ M isoproterenol and 5.0 mM [Ca$^{2+}$]o. *p<0.05 and **p<0.01 vs. baseline. Data are mean±SEM. Note that dT/dt increased with increasing [Ca$^{2+}$]o in all three groups but failed to increase with isoproterenol in SHR-NF and SHR-F.} \]
(10^-6 M) is plotted in Figure 7. With 5.0 mM [Ca^{2+}]_o, (Figure 7a), both peak light and dT/dt increased in the WKY and both SHR groups; these increases appeared to be more prominent in the SHR than in the WKY group. These data suggest that [Ca^{2+}]_o release is augmented in the SHR relative to the WKY group and that the inotropic response is proportional to the magnitude of the calcium transient. As with increased [Ca^{2+}]_o, the aequorin signal increased in response to 10^-6 M isoproterenol in all three groups (Figure 7b). In the WKY group, the augmented peak [Ca^{2+}]_o appears to be associated with a small but significant increase in inotropy (p<0.05). Despite the increase in peak [Ca^{2+}]_o, 10^-6 M isoproterenol did not result in augmented inotropy in either of the SHR groups.

Changes in the effect of isoproterenol (10^-6 M) on the temporal aspects of the mechanical contraction and the aequorin signal are presented in Figure 8. Isoproterenol administration abbreviated L_1/2 in both SHR groups (p<0.01), whereas the change in the WKY group did not achieve statistical significance. These changes are in contrast to those seen with TPL, where no effect of isoproterenol was seen. The differences in TPL between the SHR-F and WKY groups under basal conditions persisted after isoproterenol administration, and a significant difference between the WKY and SHR-NF groups was noted. Both TPT and RT_1/2 were significantly abbreviated by isoproterenol in both SHR groups (p<0.01); this effect did not reach statistical significance in the WKY group. Baseline statistical differences in TPT between both SHR and the WKY groups persisted after isoproterenol administration.

Data on fractional luminescence and estimated intracellular calcium (with 1.0 mM [Ca^{2+}]_o at 30°C and a stimulation rate of 0.33 Hz) are presented in Table 2. Diastolic intracellular calcium was significantly lower in the SHR-F group relative to the WKY group (p<0.01) and was lower in the SHR-F group

**FIGURE 6.** Bar graphs showing response of the calcium transient to [Ca^{2+}]_o and isoproterenol in three rat groups: normotensive Wistar-Kyoto rats (WKY), spontaneously hypertensive rats without heart failure (SHR-NF), and spontaneously hypertensive rats with heart failure (SHR-F). Panel a: Peak light (control is 1.25 mM [Ca^{2+}]_o) for 0.625, 1.25, 2.5, and 5.0 mM [Ca^{2+}]_o. *p<0.05 and **p<0.01 vs. 1.25 mM [Ca^{2+}]_o. Panel b: Peak light in the baseline state (no isoproterenol) with 10^-8, 10^-7, and 10^-6 M isoproterenol and with 10^-6 M isoproterenol and 5.0 mM [Ca^{2+}]_o. *p<0.05 and **p<0.01 vs. baseline. Data are mean±SEM. Note that peak light increased with both 5.0 mM [Ca^{2+}]_o, and with isoproterenol in the WKY and both SHR groups.

**FIGURE 7.** Plots showing relation between relative inotropy (dT/dt) and relative peak light with 5.0 mM [Ca^{2+}]_o (panel a) and 10^-6 M isoproterenol (panel b) in three rat groups: normotensive Wistar-Kyoto rats (WKY), spontaneously hypertensive rats without heart failure (SHR-NF), and spontaneously hypertensive rats with heart failure (SHR-F). Data are mean±SEM; values are normalized by control values (1.25 mM [Ca^{2+}]_o). Note that with 5.0 mM [Ca^{2+}]_o, both peak light and dT/dt increased in all groups. With isoproterenol, in contrast, peak light increased in all groups, but dT/dt failed to increase in the SHR-NF and SHR-F groups.
than in the SHR-NF group ($p<0.05$). $[\text{Ca}^{2+}]$, measured at the peak of the calcium transient did not differ among groups.

Myosin isozyme data are presented in Table 3. There was a significant shift in myosin isozyme profile from $V_1$ to $V_3$ in both SHR groups relative to the WKY group, with a further shift toward $V_3$ in the SHR-F group. In fact, only a single peak ($V_3$) was detected on the gel scan in five muscle preparations analyzed from the SHR group with evidence suggesting heart failure.

**Discussion**

After a prolonged period of stable hypertrophy, many SHR between the ages of 18 and 24 months develop a constellation of findings that suggest impaired left ventricular function. Furthermore, left ventricular papillary muscles from these SHR demonstrate depression of contractile function, suggesting that impaired muscle function underlies this impairment of left ventricular function. One of the objectives of studying two groups of SHR, one with and one without findings suggestive of heart failure, was to identify events that may be associated with the development of impaired myocardial function. In the present study, SHR-F and SHR-NF groups both demonstrate depression of papillary muscle function in comparison with age-matched WKY rats. These observations, together with those from earlier studies, which suggest a transition in muscle function between 18 and 24 months in SHR, are consistent with the concept that the “failure state” may not represent an abrupt transition in muscle function but rather a portion of a continuum in which many older SHR have impaired myocardial function before findings suggestive of overt failure develop. The clinicopathological distinction between the SHR groups may not be adequate to distinguish rats with normal myocardium from those with abnormal myocardium.

Since intracellular calcium handling has been implicated as a possible cause for myocardial dysfunction in a number of settings, we were interested in examining mechanical performance and intracellular calcium transients in myocardium from SHR that had developed findings suggestive of myocardial dysfunction. TPL was significantly prolonged in the SHR-F group. Gwathney and Morgan, in the pressure-overloaded ferret right ventricle, reported a delay in the decline of the calcium transient without an increase in TPL. Although it may be difficult to compare right ventricular pressure overload in the

**FIGURE 8.** Bar graphs showing temporal responses of calcium transient and tension development to isoproterenol ($10^{-6}$ M) in three rat groups: normotensive Wistar-Kyoto rats (WKY), spontaneously hypertensive rats without heart failure (SHR-NF), and spontaneously hypertensive rats with heart failure (SHR-F). Panel a: Time to peak light (TPL). Panel b: Time from peak to 50% fall in light ($L_{1/2}$). Panel c: Time to peak tension (TPT). Panel d: Time from peak to 50% fall in active tension ($R_{1/2}$). Data are mean±SEM. *$p<0.05$ and **$p<0.01$ (group effect); ###$p<0.01$ (isoproterenol vs. baseline).

**TABLE 2.** Aequorin Light Signal Intensity and Calculated $[\text{Ca}^{2+}]$.

<table>
<thead>
<tr>
<th>Diastolic calcium level</th>
<th>Peak of calcium transient</th>
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<tbody>
<tr>
<td>$L/L_{\text{max}}$</td>
<td>$[\text{Ca}^{2+}]$</td>
</tr>
<tr>
<td>(×10^6)</td>
<td>(μM)</td>
</tr>
<tr>
<td>WKY ($n=6$)</td>
<td>$8.6±0.8$ $0.38±0.02$</td>
</tr>
<tr>
<td>SHR-NF ($n=8$)</td>
<td>$6.5±0.7$ $0.32±0.02$</td>
</tr>
<tr>
<td>SHR-F ($n=4$)</td>
<td>$3.8±0.5^\dagger$ $0.23±0.02^\ddagger$</td>
</tr>
</tbody>
</table>

Values are mean±SEM. WKY, normotensive Wistar-Kyoto rats; SHR-NF, spontaneously hypertensive rats without heart failure; SHR-F, spontaneously hypertensive rats with heart failure.

$^*p<0.01$ vs. corresponding value for WKY; $^\dagger p<0.05$ vs. corresponding value for SHR-NF.

**TABLE 3.** Myosin Isozymes.

<table>
<thead>
<tr>
<th></th>
<th>$V_1$ (%)</th>
<th>$V_2$ (%)</th>
<th>$V_3$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY ($n=7$)</td>
<td>$14.1±2.6$</td>
<td>$21.5±2.3$</td>
<td>$64.4±4.8$</td>
</tr>
<tr>
<td>SHR-NF ($n=9$)</td>
<td>$8.80±1.6$</td>
<td>$14.8±1.4$*</td>
<td>$76.4±2.7^\ddagger$</td>
</tr>
<tr>
<td>SHR-F ($n=5$)</td>
<td>$0.0±0.0^\ddagger$</td>
<td>$0.0±0.0^\ddagger$</td>
<td>$100.0±0.0^\ddagger$</td>
</tr>
</tbody>
</table>

Values are mean±SEM. WKY, normotensive Wistar-Kyoto rats; SHR-NF, spontaneously hypertensive rats without heart failure; SHR-F, spontaneously hypertensive rats with heart failure.

$^*p<0.01$ and $^\ddagger p<0.05$ vs. corresponding value for WKY; $^\dagger p<0.05$ and $^\ddagger p<0.01$ vs. corresponding value for SHR-NF.
ferret with left ventricular overload with failure in the rat, the general finding of a prolonged calcium transient may be consistent with the concept that a prolongation of the calcium transient reflects impaired calcium handling in cardiac hypertrophy.

Quantitative determinations of peak systolic [Ca\(^{2+}\)]\(_{\text{in}}\) in rat papillary muscles have been reported in the range of 1.3–3.2 \(\mu\)M with a bath calcium of 2.0 mM and a stimulation rate of 0.2 Hz.\(^{20}\) The present studies were carried out at a 0.33-Hz stimulation rate in a bath containing 1.0 mM calcium. Thus, the negative staircase response in the rat (which is presumably associated with a lower [Ca\(^{2+}\)]\(_{\text{in}}\)), as well as the decreased [Ca\(^{2+}\)]\(_{\text{in}}\) may partly explain our finding of peak [Ca\(^{2+}\)], achieved during calcium transients in the range of 0.52–0.61 \(\mu\)M. Our resting intracellular calcium values in the range of 0.23–0.38 \(\mu\)M are similar to those reported in rat ventricular myocytes using acquirin (0.18–0.79 \(\mu\)M).\(^{27,28}\) Resting [Ca\(^{2+}\)]\(_{\text{in}}\)s were lower in the SHR-F group relative to the WKY (\(p<0.01\)) and SHR-NF (\(p<0.05\)) groups. The low resting [Ca\(^{2+}\)]\(_{\text{in}}\) in the SHR-F group may reflect intracellular calcium depletion and may be a factor that contributes to depressed mechanical performance observed in the baseline state. On the other hand, the preserved response of the intracellular calcium transient and inotropy to 5.0 mM [Ca\(^{2+}\)]\(_{\text{in}}\) suggests that the depressed mechanical performance observed in both SHR groups is not due to depression of calcium influx or diminished responsiveness to calcium. The latter is consistent with skinned fiber data from these rats, in which we have observed no differences in myofilament calcium sensitivity among the three groups studied.\(^{29}\) These data suggest that intracellular calcium mobilization in the SHR is intact, whereas the inotropic response appears generally proportional to the increase in intracellular calcium. Thus, the responses to calcium-mediated inotropic interventions appear to be preserved in failing hearts. The present findings are consistent with isolated muscle data indicating that interventions that increase intracellular calcium are effective in failing hearts (e.g., digitalis glycosides and postextrasystolic potentiation).\(^{30}\)

Although others\(^{30,31}\) have suggested impairment of the \(\beta\)-adrenergic system in cardiac failure, we find no depression of the calcium transient (in the present study) or of the lusitropic response to isoproterenol\(^{32}\) in the SHR in comparison with control rats. In a previous study,\(^{33}\) we found that administration of forskolin to muscle preparations from both groups of SHR elicited a lusitropic effect (in the absence of an inotropic effect, as was observed in the WKY group) that was similar to that of isoproterenol. Thus, coupling of \(\beta\)-adrenergic stimulation and cAMP formation appears functionally intact in both SHR groups studied. These findings contrast with those from studies in human myocardium that suggest deficient cAMP production in end-stage heart failure.\(^{34}\) In addition to differences in species and the model of heart failure studied, the possibility that quantitative changes in \(\beta\)-adrenergic responses are present is not excluded, although the lusitropic concen-

tration–response relation to isoproterenol appears similar in WKY and SHR groups.\(^{32}\)

In contrast to the effects of increased [Ca\(^{2+}\)]\(_{\text{in}}\), isoproterenol produces a decline in peak tension in both SHR groups (see Figure 4). The assessment of inotropy after isoproterenol addition is complicated by the abbreviation of contractile activation caused by this agent. Because of the opposing effects of isoproterenol on peak tension, we have evaluated inotropy by measuring dT/dt.\(^{26}\) Although peak [Ca\(^{2+}\)] increases in response to isoproterenol in all groups, preparations from both SHR groups failed to demonstrate an increase in inotropy; that is, dT/dt did not increase. An equivalent increase in peak [Ca\(^{2+}\)] in the WKY group, on the other hand, significantly augmented inotropy. In a more recent study\(^{35}\) of the rat (WKY) with chronic aortic constriction, we have similarly observed the absence of an inotropic response to isoproterenol while responsiveness to [Ca\(^{2+}\)], remained intact; studies of intracellular calcium have not yet been carried out in this model. These findings in the aortic-banded model suggest that the change in responsiveness to isoproterenol is related to the effects of pressure overload rather than to a response specific to the SHR.

Kurihara and Konishi\(^{36}\) have studied the effects of \(\beta\)-adrenergic stimulation on the calcium transients and mechanical activity in rat heart muscle. They suggest that when intracellular calcium stores are high, muscle preparations are nearly fully activated and relatively insensitive to the inotropic effects of catecholamines. Extrapolating their findings to our data demonstrating absent inotropic responses of both SHR groups to isoproterenol might suggest that SHR preparations are functioning on a higher portion of the pCa–tension relation than the WKY. This is not consistent, however, with our data demonstrating both augmented calcium transients and inotropy in response to increased [Ca\(^{2+}\)]\(_{\text{in}}\) in these same preparations. It should be pointed out that it was necessary to study calcium concentration–response relations before studies with isoproterenol. Thus, although all groups were allowed a similar recovery time after the change from 5.0 to 1.0 mM [Ca\(^{2+}\)]\(_{\text{in}}\), it is possible that SHR hearts may have remained calcium loaded after treatment with 5.0 mM [Ca\(^{2+}\)]\(_{\text{in}}\). This appears unlikely, however, because dT/dt and tension had fallen to baseline values before the isoproterenol studies.

We found, as did Kurihara and Konishi,\(^{36}\) that TPL was not shortened by isoproterenol (see Figure 8); they suggested that the calcium release rate is not affected by catecholamines. On the other hand, we found (as did Kurihara and Konishi) that the decay of the calcium transient was abbreviated by isoproterenol, suggesting enhanced calcium uptake. If baseline differences in TPL among groups reflect differences in the calcium release rate, our findings suggest slower calcium release rates in the SHR-F group. Kurihara and Konishi suggest that the rate of calcium release may be determined by a membrane excitation process. This explanation might be consistent with
our data demonstrating a prolongation of the electromechanical delay time in myocardial preparations from hearts with evidence of failure.5

The observed effects of isoproterenol on the calcium transient and on relaxation are consistent with catecholamine-induced cAMP release. Norepinephrine has been shown to increase the inward calcium current by increasing the probability that the calcium channel will be open.37 This is consistent with the increased calcium transient seen in all groups of rats in the present study after administration of isoproterenol. cAMP also stimulates calcium uptake by the cardiac relaxing system,38 and phosphorylation of tropolin 1 by cAMP-dependent protein kinase has been reported to enhance the calcium “off-rate” from troponin C.39 These actions may both enhance relaxation as seen in the present experiments in all groups of rats studied.

The finding of absent inotropy as manifested by unchanging dT/dt and a decline in isometric tension after isoproterenol administration in both SHR groups despite the presence of an augmented calcium transient contrasts with the intact inotropic effect seen in the WKY group. One might hypothesize that the absence of inotropy with isoproterenol observed in SHR muscle preparations may be due to limitations in energy supply and that the increased metabolic demands imposed by isoproterenol result in substrate depletion or metabolite accumulation, either of which may depress contractile performance. We have previously found that isoproterenol administration during hypoxia results in transient inotropy with a subsequent fall in active tension and the development of contracture40; no such pattern was observed in the present study. In addition, we have found impaired baseline performance in the isolated SHR left ventricle without evidence of lactate production,41 suggesting that depressed pressure occurs without hypoxia or ischemia. Thus, absent inotropy with isoproterenol is not readily explained by metabolic factors.

Another mechanism that must be considered is that of altered calcium sensitivity of the contractile apparatus. It is well recognized that catecholamines such as isoproterenol enhance [Ca2+]i to a greater extent than mechanical performance; thus, β-adrenergic stimulation shifts the force–pCa relation to the right.26 The mechanism for the decrease in tension may include troponin I phosphorylation, which results in decreased affinity of troponin C for calcium and an abbreviated crossbridge attachment time.39 This effect might be enhanced in the SHR.

Another possible explanation may be related to the shift in myosin isozyme distribution in rat myocardium from the V1 to the V3 isoform, as has been reported in a number of studies of cardiac hypertrophy in the rat.42,43 Studies of the aging SHR have shown progressive shifts from the V1 to the V3 isoform of myosin,44 and in the present study, we have observed that left ventricular papillary muscles from the SHR-F contain virtually 100% V3 myosin (Table 3). Crossbridge cycling rate, as measured by perturbation analysis, has been reported to be two-fold higher in V1 as compared with V3 muscle.45 Using this technique, Hoh et al.46 reported that adrenaline increases crossbridge cycling rate to a greater degree in myocardium containing predominantly V1 as compared with V3 myosin. These findings are generally consistent with those of Winegrad and Weisberg,47 who found that cAMP increases ATPase activity of rat myocardium containing V1 myosin, whereas ATPase activity is inhibited in the case of V3 myosin. If inotropy after a cAMP-mediated intervention such as isoproterenol is due to an increase in the crossbridge cycling rate (as well as increased [Ca2+]i), absent inotropy may be related, in part, to a shift in myosin isozyme distribution to V3, where crossbridge cycling increases little or not at all. Morano et al.48 studying the stroke-prone SHR, also report shifts in myosin isozyme distribution that progress with aging and occur earlier in male than in female stroke-prone SHR. They suggest a relation between myosin isozyme shifts and inotropic responsiveness of the contractile apparatus. Thus, the predominance of V3 myosin may contribute to the absent inotropic response to isoproterenol seen in the two SHR groups.

In summary, important changes in [Ca2+]i, and mechanical activity are observed during the transition to heart failure in the SHR. It remains to be determined if these changes are primary events associated with the development of left ventricular decompensation or secondary events suggesting adaptation of the overloaded myocardium.

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