Effects of Treppe and Calcium on Intracellular Calcium and Function in the Failing Heart From the Spontaneously Hypertensive Rat

Wesley W. Brooks, Oscar H.L. Bing, Sheldon E. Litwin, Chester H. Conrad, James P. Morgan

Abstract We studied functional and intracellular calcium responses to treppe and extracellular calcium in spontaneously hypertensive rat (SHR) hearts during the transition from compensated pressure overload to failure. Intracellular calcium was measured using aequorin, a bioluminescent Ca\(^{2+}\) indicator. Experiments were performed with intact, isovolumically contracting, buffer-perfused hearts from three rat groups: (1) aging SHR with evidence of heart failure (SHR-F), (2) age-matched SHR with no evidence of heart failure (SHR-NF), and (3) age-matched normotensive Wistar-Kyoto (WKY) rats. In each experiment, left ventricular performance and intracellular calcium transients were simultaneously recorded. Hearts were studied at 30°C and paced at a rate of 1.6 Hz while being perfused with oxygenated Krebs-Henseleit solution (95% O\(_2\)/5% CO\(_2\)) at 100 mm Hg. At the baseline state, peak systolic pressure was greatest in the SHR-NF group and lowest in the SHR-F group. Peak and resting [Ca\(^{2+}\)]\(_i\) were not significantly different among groups; however, the calcium transient was prolonged in the SHR-NF and SHR-F groups. With increasing perfusate [Ca\(^{2+}\)]\(_o\) from 0.5 to 3.0 mmol/L, the relative increases in peak [Ca\(^{2+}\)]\(_i\), from 0.5 to 3.0 mmol/L, the relative increases in peak [Ca\(^{2+}\)]\(_i\), and peak systolic pressure were similar among groups. When stimulation rate was increased from 1.6 to 2.0, 2.4, 2.8, and 3.2 Hz, peak [Ca\(^{2+}\)]\(_i\), peak systolic pressure, and ±dp/dt fell in SHR-F hearts. Peak systolic pressure decreased in the SHR-NF group at rates above 2.4 Hz but did not decline in the WKY group. Peak [Ca\(^{2+}\)]\(_i\), increased in the WKY and SHR-NF groups with increasing heart rates. Peak systolic pressure did not fall significantly in the WKY and SHR-NF hearts. We conclude the following: (1) Under baseline conditions, depressed contractile function of failing myocardium cannot be explained by decreased peak [Ca\(^{2+}\)]\(_i\); (2) relative increases in [Ca\(^{2+}\)]\(_o\) and inotropy with increasing [Ca\(^{2+}\)]\(_o\) are proportional among groups; and (3) although peak systolic [Ca\(^{2+}\)]\(_i\), and inotropy are maintained with increasing stimulation rate in the WKY and SHR-NF groups, peak systolic [Ca\(^{2+}\)]\(_i\), and pressure decrease in parallel in the SHR-F heart with increasing stimulation rate, suggesting that impaired calcium cycling may contribute to compromised pump function in the SHR-F heart. (Hypertension. 1994;24:347-356.)

Key Words • heart hypertrophy • heart failure, congestive • calcium • muscle, cardiac
preparations, thus avoiding potentially confounding variables such as core hypoxia that may be present in isolated papillary muscle preparations at higher stimulation frequencies. We considered that by comparing two interventions which increase both energy demands and the demands on intracellular calcium control systems, we may be able to obtain additional insights into possible mechanisms of cardiac decompensation in the SHR. Therefore, in the present study we investigated the effects of perfusate calcium concentration and stimulation rate on intracellular calcium handling and left ventricular function in the SHR model of chronic left ventricular pressure overload and failure.

Methods

Animal Model

Male SHR and normotensive Wistar-Kyoto (WKY) control rats, 6 to 9 months of age, were purchased as retired breeders from Taconic Farms, Germantown, NY, and boarded in the animal facility at the Boston VA Medical Center until the time of study (18 to 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, at 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 to 2 weeks. No WKY rats developed respiratory difficulties. A few SHR with respiratory findings died before they could be studied and at autopsy had pathological findings consistent with heart failure, as described below. Isolated intact perfused heart preparations were examined from three rat groups: (1) aging SHR with physical and physiological 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.

Cardiac catheterization was performed on a number of animals from each group on the day of study as previously described. Briefly, animals were anesthetized with ether, and the right carotid artery was isolated. A catheter-tipped micromanometer (model SPR-249, Millar Instruments) was advanced into the left ventricle where left ventricular pressures were recorded at high and low gain in order to obtain baseline hemodynamic data.

Preparation of Isolated Perfused Heart

The isolated isovolumically contracting rat heart preparation has been previously described. Briefly, at the time of study the rats were decapitated. Their hearts were quickly removed and placed in oxygenated bicarbonate-buffered physiological salt solution containing heparin at 23° to 25°C. The ascending aorta was cannulated, the pulmonary artery opened, and hearts were perfused with oxygenated Krebs-Henseleit solution at 30°C with a constant flow set to achieve a pressure of approximately 100 mm Hg. Pressure in the aortic root was measured by a sidearm cannula connected to a pressure transducer. The composition of the perfusate (mmol/L) was NaCl 120, KCl 5.9, NaHCO3 25, NaH2PO4 1.2, MgCl2 1.2, CaCl2 1.0, and dextrose 11.5. The perfusate was bubbled with a gas consisting of 95% O2 and 5% CO2, resulting in a pH of 7.4 and PO2 of approximately 200 mm Hg. After initiation of coronary perfusion, a small latex fluid-filled balloon was placed in the left ventricular chamber via the left atrium. The balloon was connected to a pressure transducer by a short length of polyethylene tubing for determination of left ventricular pressure. Left ventricular volume was determined as previously described by Kihara et al. 11-12 In brief, hearts were cooled to 24°C and perfused with a medium containing 0.2 mmol/L calcium. Within 2 minutes systolic pressure fell from 100 to less than 20 mm Hg. After 5 minutes of 0.2 mmol/L calcium perfusion, coronary perfusion was reduced to achieve a perfusion pressure of 10 to 20 mm Hg in preparation for aequorin loading. Subsequently, the heart was temporarily raised out of the organ bath, and aequorin solution (3 to 5 μL) was injected with a low-resistance glass micropipette into the interstitium of the epicardium of the apex of the left ventricle just beneath the epicardium. The heart was then repositioned in the bottom of the organ bath.

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. 11-12 In brief, this system consists of an ellipsoid 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) at the other. This arrangement maximized the collection of light emitted from the aequorin-loaded site.

After aequorin loading, calcium was gradually reintroduced into the perfusate, and coronary flow rate and temperature were restored to aequorin loading baseline conditions. Atrial pacing was carried out using a right atrial bipolar pacemaker wire driven by a stimulator (model S88, Grass Instruments) delivering 4-millisecond monophasic square-wave pulses. Stimulation threshold was determined by slowly increasing the voltage until consistent pacing occurred, and the voltage was set to 10% above threshold. A stimulation frequency of 1.6 Hz was used in the baseline state. After instrumentation, the heart was positioned in a temperature-controlled bath and partially submerged in solution delivered from the same bottle as the coronary perfusate.

At the conclusion of the experiment, the cardiac chambers were dissected, blotted firmly, weighed, and placed overnight into a drying oven at 55°C. All ventricular samples were reweighed after 24 hours of drying for determination of the ratio of wet to dry weight.

Hemodynamic Parameters

Baseline measurements were obtained when the preparation achieved a steady state after instrumentation (approximately 15 minutes). After this equilibration period, a baseline pressure-volume relation was determined; left ventricular peak systolic and end-diastolic pressures were measured in the oxygenated control state 20 to 30 seconds after 0.02-ml increments in balloon volume. Volumes were increased until an end-diastolic pressure of approximately 20 mm Hg was achieved. The balloon volume was then adjusted to an end-diastolic pressure of 2 to 4 mm Hg and kept isovolumic throughout the experiment.

Loading Procedure for Aequorin

After equilibration and baseline measurements, a localized region of the left ventricular apex was microinjected with aequorin as previously described. 11-13 In brief, hearts were cooled to 24°C and perfused with a medium containing 0.2 mmol/L calcium. Within 2 minutes systolic pressure fell from 100 to less than 20 mm Hg. After 5 minutes of 0.2 mmol/L calcium perfusion, coronary perfusion was reduced to achieve a perfusion pressure of 10 to 20 mm Hg in preparation for aequorin loading. Subsequently, the heart was temporarily raised out of the organ bath, and aequorin solution (3 to 5 μL) was injected with a low-resistance glass micropipette into the interstitium of the epicardium of the apex of the left ventricle just beneath the epicardium. The heart was then repositioned in the bottom of the organ bath.

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After aequorin loading, calcium was gradually reintroduced into the perfusate, and coronary flow rate and temperature were restored to aequorin loading baseline conditions while the aequorin light signal reached a steady state. At this point aequorin light signals and left ventricular isovolumic pressures were simultaneously recorded on strip-chart recording paper and magnetic tape.

Protocol

Baseline isovolumic contraction parameters were recorded simultaneously with the aequorin light signal from all heart preparations after a 10- to 15-minute equilibration period under baseline conditions. In preparation for determination of the concentration-response relation to calcium, the perfusate was replaced with a zero phosphate solution to avoid precipitation of calcium salts. Increasing concentrations of calcium were then added to the perfusate to obtain a dose-response

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TABLE 1. In Vivo Heart Rate and Hemodynamic Data

<table>
<thead>
<tr>
<th>Group</th>
<th>HR, bpm</th>
<th>SP, mm Hg</th>
<th>DP, mm Hg</th>
<th>+dP/dt, mm Hg/s</th>
<th>−dP/dt, mm Hg/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY (n=16)</td>
<td>404±48*</td>
<td>105±11†</td>
<td>3±1*</td>
<td>5444±871‡</td>
<td>3760±883</td>
</tr>
<tr>
<td>SHR-F (n=13)</td>
<td>305±35†</td>
<td>101±11†</td>
<td>10±55</td>
<td>2907±347§</td>
<td>2582±374§</td>
</tr>
<tr>
<td>SHR-NF (n=7)</td>
<td>407±43</td>
<td>138±17†</td>
<td>6±3</td>
<td>6218±167</td>
<td>4589±933</td>
</tr>
</tbody>
</table>

HR indicates heart rate; SP, systolic pressure; DP, diastolic pressure; +dP/dt, maximal rate of rise of systolic pressure; −dP/dt, maximal rate of fall of systolic pressure; WKY, normotensive Wistar-Kyoto rats; SHR-NF, spontaneously hypertensive rats without heart failure; and SHR-F, spontaneously hypertensive rats with heart failure. Values are mean±SD.

*P<.01, ‡P<.05 vs SHR-F.
†P<.01, §P<.05 vs SHR-NF.

Statistical Analysis

Data from the SHR-F, SHR-NF, and WKY groups were compared using one-way ANOVA with replication. A one-way ANOVA was used to examine group effects. The Newman-Kuels multiple-sample comparison test was used to localize differences when appropriate. Data are expressed as mean±SD.

Results

Cardiac Catheterization

Studies were conducted with 24 SHR and 16 WKY rats, 18 to 24 months of age. Fourteen SHR had findings of heart failure (SHR-F group), including tachypnea (n=13), pleural and/or pericardial effusions (n=14), left atrial thrombi (n=14), and right ventricular hypertrophy (as defined by right ventricular weight–body weight ratio >0.8 mg/g, n=14).

Cardiac catheterization was performed on 13 animals in the SHR-F group, 7 SHR-NF, and 10 WKY rats (Table 1). Tail-cuff blood pressures obtained in the conscious animal were elevated in both SHR groups (SHR-NF, 177±22 mm Hg; SHR-F, 150±13 mm Hg) relative to the WKY group (116±4 mm Hg, P<.01) but were lower in the SHR-F group compared with the SHR-NF group (P<.05, Table 2). In all groups, peak systolic measurements were lower in the anesthetized animals compared with tail-cuff measurements in the conscious animals. Peak systolic pressure was greater in the SHR-NF group compared with both the SHR-F and WKY groups. Diastolic pressure was elevated in the SHR-F group relative to the WKY and SHR-NF groups. Mean aortic (perfusion) pressure for the WKY group was 86±16 mm Hg; SHR-F, 86±20 mm Hg; and SHR-NF, 118±17 mm Hg.

Clinicopathological Data

Fibrosis was noted by gross inspection in the hearts from both the SHR-F and SHR-NF groups; fibrosis was not evident in any of the WKY hearts. Table 2 presents data on body weight, cardiac chamber weight, and chamber weight normalized for body weight. Both SHR groups demonstrated left ventricular hypertrophy, as indicated by an increase in the ratio of left ventricular weight to body weight; this ratio was greater in the SHR-F relative to the SHR-NF group. Right ventricular hypertrophy was one of the criteria for selection of the SHR-F group; the ratio of right ventricular weight to body weight increased in the SHR-F group compared with both the SHR-NF and WKY groups.

TABLE 2. Chamber Weight, Body Weight, and Blood Pressure Data

<table>
<thead>
<tr>
<th>Group</th>
<th>LV, g</th>
<th>RV, g</th>
<th>BW, g</th>
<th>LV/BW, mg/g</th>
<th>RV/BW, mg/g</th>
<th>BP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY (n=16)</td>
<td>1.4±0.2</td>
<td>0.3±0.1</td>
<td>560±92‡</td>
<td>2.6±0.4‡</td>
<td>0.6±0.3‡</td>
<td>116±4‡</td>
</tr>
<tr>
<td>SHR-F (n=16)</td>
<td>1.9±0.2</td>
<td>0.5±0.1</td>
<td>398±32</td>
<td>4.9±0.6†</td>
<td>1.2±0.1‡</td>
<td>150±13</td>
</tr>
<tr>
<td>SHR-NF (n=12)</td>
<td>1.5±0.3</td>
<td>0.2±0.03</td>
<td>415±56</td>
<td>3.6±0.8</td>
<td>0.6±0.1</td>
<td>177±22</td>
</tr>
</tbody>
</table>

LV indicates left ventricular wet weight; RV, right ventricular wet weight; BW, body weight; BP, systolic arterial pressure determined via indirect tail-cuff method; WKY, normotensive Wistar-Kyoto rats; SHR-NF, spontaneously hypertensive rats without heart failure; and SHR-F, spontaneously hypertensive rats with heart failure. Values are mean±SD.

*P<.01, †P<.05 vs SHR-F.
‡P<.05 vs SHR-NF.
pressed ($P<.01$) in the SHR-F group relative to both the SHR-NF and WKY groups.

Table 4 presents light transient and mechanical temporal data. The duration of the light transient ($TPL$, $L_{90}$, and $TPL+L_{90}$) was increased in the SHR-NF compared with the WKY group. Time to peak pressure ($TPP$) was increased in both SHR groups relative to the WKY group. The time needed for pressure to fall from peak to 50% of peak ($P_{50}$) was reduced in the SHR-F group compared with the WKY group.

**Resting and Peak Intracellular Calcium Concentrations**

Resting and peak intracellular calcium concentrations were calculated from signals in WKY, SHR-NF, and SHR-F hearts using the method of fractional luminescence. Table 5 summarizes the fractional luminescence values for each group. Data on fractional luminescence and estimated intracellular calcium with 1.0 mmol/L perfusate calcium were obtained under baseline conditions (stimulation rate, 1.6 Hz at 30°C). Diastolic intracellular calcium and the peak of the $Ca^{2+}$ transient were not significantly different among groups.

**Changing Perfusate Calcium Concentration**

Fig 3 demonstrates the effect of increasing perfusate calcium concentration on left ventricular function and the aequorin light signal. When perfusate calcium was increased from 0.5 to 3.0 mmol/L, peak systolic pressure increased in all groups (WKY, from 55±17 to 101±27 mm Hg; SHR-NF, from 75±25 to 133±30 mm Hg; SHR-F, from 41±10 to 77±21 mm Hg; all $P<.001$). Significant increases in systolic pressure and the aequorin light signal were seen in all groups in response to increasing calcium (Figs 3 and 4). The light signal response to increasing calcium in the SHR-NF and SHR-F groups appeared greater than in the WKY group. To test the calcium responsiveness of the nor-motensive, hypertrophied, and failing hearts, we determined the calcium concentration-response relation. However, the percent of maximal developed pressure and light at increasing calcium concentrations were comparable among the groups at each calcium concentration studied (Fig 5). The duration of both the pressure and light signals ($TPP$ and $TPL$) were largely unchanged at the various calcium concentrations. The relaxation parameters of the pressure and light signals tended to decrease slightly with increasing perfusate calcium. There were no significant differences in the response of these parameters among the groups at each calcium concentration examined.

**Changing Stimulation Rate**

Fig 6 demonstrates the effect of increasing stimulation rate on the aequorin light signal and left ventricular function. The left panel demonstrates the usual pattern observed in the unpaced (intrinsic heart rate) preparation under baseline conditions in the WKY, SHR-NF, and SHR-F hearts. Table 6 compares the changes in baseline aequorin light signal and left ventricular functional parameters for the three groups as the stimulation rate increased from 1.6 Hz. As noted above, the intrinsic heart rate of the SHR-F hearts was significantly slower than both SHR-NF and WKY hearts ($P<.01$). At a constant paced rate of 1.6 Hz, peak systolic pressure
TABLE 3. Comparison of Hemodynamic Parameters at Steady State

<table>
<thead>
<tr>
<th>Group</th>
<th>LV Systolic Pressure, mm Hg</th>
<th>Diastolic Pressure, mm Hg</th>
<th>LV Wall Thickness, mm</th>
<th>LV Chamber Radius, mm</th>
<th>Systolic Stress, kdyne/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY (n=10)</td>
<td>93±22*</td>
<td>3±2</td>
<td>0.46±0.04</td>
<td>0.23±0.01</td>
<td>16.0±5.6</td>
</tr>
<tr>
<td>SHR-F (n=13)</td>
<td>65±12†</td>
<td>3±2</td>
<td>0.51±0.04</td>
<td>0.26±0.04</td>
<td>11.2±3.9‡</td>
</tr>
<tr>
<td>SHR-NF (n=11)</td>
<td>120±22</td>
<td>4±3</td>
<td>0.48±0.04</td>
<td>0.24±0.04</td>
<td>20.2±6.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>LV Thickness, mm</th>
<th>LV Chamber Radius, mm</th>
<th>LV Systolic Pressure, mm Hg</th>
<th>Diastolic Pressure, mm Hg</th>
<th>LV Wall Thickness, mm</th>
<th>LV Chamber Radius, mm</th>
<th>Systolic Stress, kdyne/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY (n=11)</td>
<td>73±11†</td>
<td>84±18</td>
<td>176±19</td>
<td>137±16*</td>
<td>138±14*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR-F (n=13)</td>
<td>78±16</td>
<td>95±20</td>
<td>198±25</td>
<td>156±20</td>
<td>115±15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR-NF (n=11)</td>
<td>77±10</td>
<td>103±19‡</td>
<td>211±20§</td>
<td>166±18†</td>
<td>137±25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LV indicates left ventricular; WKY, normotensive Wistar-Kyoto rats; SHR-NF, spontaneously hypertensive rats without heart failure; and SHR-F, spontaneously hypertensive rats with heart failure. Steady-state conditions were hearts paced at 1.6 Hz at a balloon volume adjusted to an end-diastolic pressure of 2 to 4 mm Hg. Values are mean±SD.

was 94±23, 92±30, and 55±21 mm Hg in the WKY, SHR-NF, and SHR-F groups, respectively. Increasing the frequency of stimulation from a paced rate of 1.6 to 2.0 Hz resulted in a decrease in peak light and peak systolic pressure in the SHR-F hearts, whereas these parameters increased in both SHR-NF and WKY hearts. When the frequency was increased to 2.4 Hz, peak light further decreased in the SHR-F hearts and increased in the WKY and SHR-NF hearts. Peak systolic pressure was decreased in the SHR-NF hearts, markedly decreased in the SHR-F hearts, and only slightly decreased from control levels in the WKY hearts at the increased stimulation rate of 3.2 Hz. Eight of the 13 SHR-F hearts examined exhibited either light and pressure alternars or a marked increase in resting diastolic calcium and diastolic pressure values at the higher stimulation frequency. These phenomena were not in evidence in hearts from WKY or SHR-NF animals. The bottom right panel of Fig 6 demonstrates the presence of well-defined electrical alternars in the SHR-F heart at a stimulation rate of 3.2 Hz. Fig 7 is another example of the effect of increasing stimulation rate from 1.6 to 3.2 Hz in WKY and SHR-F hearts. Note the presence of distinct electrical and mechanical alternars in the SHR-F hearts and a marked increase in resting diastolic calcium and diastolic pressure values at the higher stimulation rate. As the frequency of stimulation was increased up to 3.2 Hz. In the SHR-NF hearts the amplitude of the calcium transient decreased at 2.4 Hz and decreased to a greater extent at a higher stimulation frequency. In comparison, in WKY hearts the light signal decreased more at all control values up to a frequency of 3.2 Hz. Peak light was decreased to control values in the WKY and SHR-NF groups at all stimulation rates examined.

The frequency dependence of the peak rates of pressure fall (—dP/dt) was generally similar to that pattern observed for +dP/dt. The values for —dP/dt at 1.6 Hz were 622±173, 660±221, and 416±177 mm Hg/s for the WKY, SHR-NF, and SHR-F groups, respectively. In the WKY hearts —dP/dt remained at control levels up to a frequency of 2.0 Hz. In the SHR-F hearts —dP/dt decreased at 2.4 Hz. In the SHR-NF hearts —dP/dt at 1.6 Hz were 152±14, 156±20, and 166±18† milliseconds for the WKY, SHR-NF, and SHR-F groups, respectively. At relatively low stimulation rates the SHR-F curve begins to diverge from both the SHR-NF and WKY groups. At the frequency of 2.0 Hz, peak light and +dP/dt markedly decreased in the SHR-F hearts and increased in the WKY and SHR-NF hearts (P<.01). The value for +dP/dt was first depressed relative to control values in the SHR-NF hearts at 2.4 Hz and decreased to a greater extent at a higher stimulation frequency. In comparison, in WKY hearts +dP/dt remained at control levels up to a frequency of 3.2 Hz. Peak light was decreased above control values in both the WKY and SHR-NF groups at all stimulation rates examined.

The percent of maximal rate of developed pressure (+dP/dt) and relaxation (—dP/dt) were compared at increasing stimulation rates (Fig 8). The values for +dP/dt at 1.6 Hz were 1520±400, 1109±250, and 732±353 mm Hg/s for the WKY, SHR-NF, and SHR-F groups, respectively. At relatively low stimulation rates the SHR-F curve begins to diverge from both the SHR-NF and WKY groups. At the frequency of 2.0 Hz, peak light and +dP/dt markedly decreased in the SHR-F hearts and increased in the WKY and SHR-NF hearts (P<.01). The value for +dP/dt was first depressed relative to control values in the SHR-NF hearts at 2.4 Hz and decreased to a greater extent at a higher stimulation frequency. In comparison, in WKY hearts +dP/dt remained at control levels up to a frequency of 3.2 Hz. Peak light was increased above control values in both the WKY and SHR-NF groups at all stimulation rates examined.

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Increasing the stimulation rate resulted in an abbreviation of the pressure response and to a lesser extent the duration of the intracellular calcium transient in all groups. Time to peak pressure at 1.6 Hz was 141±15, 149±24, and 152±14 milliseconds for the WKY, SHR-F, and SHR-NF groups, respectively. At 3.2 Hz these values decreased to 125±9, 120±8, and 122±15 milliseconds, respectively. In general, the duration of the light signal (TPL+L*) decreased (range, 4% to 9% among groups) in all groups when the stimulation rate was increased from 1.6 to 3.2 Hz.

TABLE 4. Temporal Characteristics of Calcium Transients and Developed Pressure Under Baseline Conditions

<table>
<thead>
<tr>
<th>Group</th>
<th>TPL</th>
<th>TPL+L*</th>
<th>Total</th>
<th>TPP</th>
<th>P1/2</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY (n=11)</td>
<td>73±11†</td>
<td>84±18</td>
<td>176±19</td>
<td>137±16*</td>
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<td>SHR-NF (n=11)</td>
<td>77±10</td>
<td>103±19†</td>
<td>211±20†</td>
<td>166±18†</td>
<td>137±25</td>
</tr>
</tbody>
</table>

TPL indicates time to peak light; TPL+L*, time to 90% decline from peak light; Total, total duration of light transient; TPP, time to peak systolic pressure; P1/2, time for pressure to fall from peak to 50% of peak; WKY, normotensive Wistar-Kyoto rats; SHR-NF, spontaneously hypertensive rats without heart failure; and SHR-F, spontaneously hypertensive rats with heart failure. Values are mean±SD and expressed as milliseconds.

*P<.05 vs SHR-F.
†P<.05 vs WKY.
TABLE 5. Resting and Peak [Ca$$^{2+}$$].

<table>
<thead>
<tr>
<th>Group</th>
<th>Resting Calcium</th>
<th>Peak Calcium</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY (n=9)</td>
<td>0.33±0.20</td>
<td>0.87±0.21</td>
</tr>
<tr>
<td>SHR-F (n=12)</td>
<td>0.24±0.13</td>
<td>0.93±0.17</td>
</tr>
<tr>
<td>SHR-NF (n=10)</td>
<td>0.26±0.17</td>
<td>0.89±0.20</td>
</tr>
</tbody>
</table>

WKY indicates normotensive Wistar-Kyoto rats; SHR-NF, spontaneously hypertensive rats without heart failure; and SHR-F, spontaneously hypertensive rats with heart failure. Values are mean±SD and expressed as micromoles per liter.

Discussion

The present study examines the relation between left ventricular pump function and intracellular calcium transients in isolated perfused hearts from SHR with compensated hypertrophy and failure relative to a non-hypertensive age-matched group of WKY hearts. Left ventricular developed pressure in the SHR-F group was significantly less than in both the SHR-NF and WKY groups. Abnormal calcium handling has been implicated as a possible cause for myocardial dysfunction in a number of settings. In the baseline state, significant differences in peak or resting [Ca$$^{2+}$$] were not present among the three animal groups studied. However, the calcium signals of the SHR-NF and SHR-F were prolonged, consistent with previous findings from isolated muscle studies of hypertrophy and failure in which depressed contractile function has been associated with prolonged calcium transients.

Since peak [Ca$$^{2+}$$], was not significantly different among the groups, decreased peak [Ca$$^{2+}$$], cannot account for the depressed left ventricular function seen in the decompensated SHR hearts. However, findings of the present study in perfused hearts show that increasing stimulation rate markedly reduced both peak light and peak systolic pressure in the SHR-F group. In the normotensive WKY group, peak light remained above control values at all stimulation rates studied, and peak systolic pressure was maintained. The response of the SHR-NF hearts was intermediate between the WKY and the SHR-F hearts. In response to increasing rates of stimulation, the maximal rate of pressure fall (−dP/dt) was most depressed in the SHR-F and to a lesser extent the SHR-NF heart relative to WKY hearts. It appears that as the heart rate is increased, the left ventricle of SHR-F hearts is unable to maintain calcium homeostasis, further exacerbating contractile dysfunction. Reduced sarcoplasmic reticulum (SR) calcium accumulation and subsequent release is thought to lead to impaired mechanical relaxation and reduced pressure development in subsequent contractions, a problem that may be enhanced at increased stimulation rates. This formulation could explain impaired SR calcium uptake and depressed function in the failing heart at rapid stimulation rates while adequate calcium handling appears to be present in the baseline state.

As noted above, the finding of similar peak [Ca$$^{2+}$$], among normotensive, hypertrophied, and failing groups...
Fig 5. Line graphs show concentration response to changes in extracellular calcium in aequorin-loaded whole hearts from normotensive Wistar-Kyoto (○) rats and spontaneously hypertensive rats without (□) and with (■) evidence of failure. Left, isovolumic pressure response expressed as percent of pressure at 3.0 mmol/L calcium concentration; right, peak light response represented as percent of light at 3.0 mmol/L calcium concentration. LV indicates left ventricular. Data are mean±SD.

(Table 5) indicates that the availability of intracellular calcium for activation of the myofilaments is not diminished. Therefore, decreased peak [Ca2+] cannot account for the depressed developed pressure of the SHR-F group in the baseline state. Peak intracellular calcium concentrations from isolated cell, papillary muscle, and perfused heart preparations have been reported to approximate 1 μmol/L [Ca2+] in normotensive animals under similar baseline experimental conditions.11,12,17,26-28 Similar to the present study, values reported from isolated rat papillary muscles of SHR-NF and SHR-F hearts and experimentally infarcted WKY hearts also indicate no significant differences in peak [Ca2+].4,17 Resting [Ca2+], was also similar for the three groups and fell within the range of 0.1 to 0.3 μmol/L [Ca2+], reported for rat ventricular myocytes using aequorin29,30 and fura 228 as well as in rat papillary muscles using aequorin.4 Findings are also comparable to resting values reported for normal and hypertrophied ferret hearts.11-13

The possibility that the responsiveness of the myofilaments to calcium is altered by hypertrophy and failure must also be considered. Although baseline left ventricular pressure was significantly reduced in the SHR-F hearts, the relative responses of light and pressure to changes in [Ca2+], were similar in normotensive, hypertrophied, and failing hearts. The similarity of these relations among groups suggests a similar responsiveness to calcium. In a study of saponin-skinned myocardial fibers from a similar group of animals, myofilament calcium sensitivity and cooperativity were not different among groups; however, maximal calcium-activated force was increased in the SHR-NF group. Maximal calcium-activated force was found to fall to control (WKY) levels with the development of failure.3 This finding in skinned myocardial fibers supports the finding of the present study that tension development is depressed with heart failure in the isolated heart. The decrease in maximal force development by the failing heart may be caused by some other factor not directly examined, such as the presence of connective tissue.31,32 Overall, depressed contractile function does not appear to be explained by a decrease in [Ca2+], or a decrease in the responsiveness of the myofilaments to calcium.

It may be considered that the increased demands associated with an increase in stimulation rate may have shifted energy supply/demand relations in favor of hypoxia or ischemia in the failing heart. However, the increased demands associated with increasing bath (and intracellular) calcium did not result in a reduced response to [Ca2+], and pressure development in the
TABLE 6. Effect of Increasing Stimulation Rate on Light and Pressure Parameters

<table>
<thead>
<tr>
<th>Stimulation Rate, Hz</th>
<th>2.0</th>
<th>2.4</th>
<th>2.8</th>
<th>3.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>PL</td>
<td>PSP</td>
<td>PL</td>
<td>PSP</td>
</tr>
<tr>
<td>WKY (n=9)</td>
<td>108±20</td>
<td>102±15</td>
<td>113±21*</td>
<td>90±8*</td>
</tr>
<tr>
<td>SHR-F (n=13)</td>
<td>95±11</td>
<td>85±10</td>
<td>87±13†</td>
<td>71±14</td>
</tr>
<tr>
<td>SHR-NF (n=6)</td>
<td>149±46</td>
<td>104±21</td>
<td>114±21</td>
<td>83±7</td>
</tr>
</tbody>
</table>

PL indicates peak light; PSP, peak systolic pressure; WKY, normotensive Wistar-Kyoto rats; SHR-NF, spontaneously hypertensive rats without heart failure; and SHR-F, spontaneously hypertensive rats with heart failure. Values are mean±SD and expressed as percent control at 1.6 Hz.

*P<.05, †P<.01 vs SHR-F.
‡P<.05, ‡P<.01 vs SHR-NF.

SHR-F group. The changes in the calcium signal in the SHR-F group with increasing heart rate are not consistent with those changes observed with either brief periods of ischemia or hypoxia. In this case, the calcium transient is relatively preserved while force falls precipitously. Smith and Allen reported no change in [Ca^{2+}] with simulated ischemia while force declined. Findings of proportional decreases in [Ca^{2+}] and pressure may be more consistent with primary impairment of SR calcium cycling with increasing heart rate as opposed to energy supply/demand relations. Additional evidence of impaired SR function found in the present study is the appearance of alternans of both calcium transients and mechanical function found only in the SHR-F group at increased stimulation rates (Fig 7). In fact, nearly 62% (8 of 13) of the hearts in the SHR-F group demonstrated an elevation of diastolic [Ca^{2+}] and/or calcium transients and pressure alternans. Although the mechanism underlying alternans is not completely understood, alternations in contractile force are thought to result from variations in intracellular calcium release. Additional evidence of impaired SR function at increasing stimulation rates with heart failure reported by other investigators are fusion of light transients and contraction with increases in both end-diastolic intracellular calcium concentration and end-diastolic tone and a decrease in peak active tension development. Under the same conditions, control muscle preparations did not exhibit fusion or a decline in the force of contraction.1,2

Myotherm data from human hearts indicate a decrease in tension-independent heat thought to be caused in part by reductions in calcium release and the rate of calcium removal. Mulieri et al have studied the effects of heart rate in isolated muscle preparations from normal and failing human hearts and found that in contrast to normal myocardium, force falls at increasing heart rates in failing myocardium; they propose that impairment of function at increasing heart rates reflects an impairment of calcium cycling.

In studies of SR function in partially skinned fibers (40 µg/mL saponin concentration for 20 minutes) from hypertrophied hearts caused by constriction of the abdominal aorta for 6 to 8 weeks, quantitative differences in responses to caffeine and inositol, 1,4,5-trisphosphate and in the ability of the SR to accumulate and release calcium and contract have led investigators to conclude that depressed calcium accumulation by the SR may play a role in the modulation of contractile performance in this model of pressure-overload hypertrophy. A similar conclusion was reached in more recent studies of SR function from chemically skinned myocardial fibers obtained from patients with idiopathic dilated cardiomyopathy and...
 congestive heart failure due to myocardial infarction in rat.\textsuperscript{37}

In summary, the present study carried out in isolated perfused hearts demonstrates depressed left ventricular function in failing SHR hearts, whereas peak intracellular calcium levels do not differ from those in control hearts. In response to an increase in perfusate calcium, peak left ventricular pressure increases in proportion to the increase in $[Ca^{2+}]$, in all groups. In contrast, at increasing heart rates, $[Ca^{2+}]$ and pressure decline at higher heart rates in the SHR-NF group. In the SHR-F group, both $[Ca^{2+}]$, and pressure markedly fall at all increases in heart rate, with the appearance of light and mechanical alternans in many hearts at the higher rates. Thus, the failing heart appears unable to increase peak intracellular calcium in response to increasing rate. Although these data may be consistent with a decreased ability of the SR to take up and release calcium at increased stimulation rates, the role of changes in calcium handling in the progressive development of mechanical dysfunction during the transition from compensated hypertrophy to heart failure remains to be further elucidated.

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References


Fig 8. Line graphs show effect of increasing stimulation frequency on maximal rate of rise and fall of systolic pressure $(\pm dP/dt)$. Data are expressed as percent change of response from 1.6 Hz. Left, $+dP/dt$ response; right, $-dP/dt$ response to increasing stimulation rates in Wistar-Kyoto (○) hearts and hypertrophied (△) and failing (●) spontaneously hypertensive rat hearts. Data are mean±SD.


Effects of treppe and calcium on intracellular calcium and function in the failing heart from the spontaneously hypertensive rat.
W W Brooks, O H Bing, S E Litwin, C H Conrad and J P Morgan

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