Diminished Tolerance of Prehypertrophic, Cardiomyopathic Syrian Hamster Hearts to Ca\(^{2+}\) Stresses

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Although abnormal myocardial calcium homeostasis in the cardiomyopathic hamster (CMH) has been documented in the hypertrophic stage of the disease, the Ca\(^{2+}\) tolerance before the hypertrophic stage has not been investigated. We studied isovolumic contractile function in response to a variety of Ca\(^{2+}\) stresses including increases in perfusate [Ca\(^{2+}\)] (Ca\(_o\)), the Ca\(^{2+}\) channel agonist Bay K 8644, and \(\alpha\)- or \(\beta\)-adrenergic agonists of isolated perfused hamster hearts from 24-45-day-old male CMH, BIO 14.6 strain, and age- and sex-matched F1B strain controls. The coronary flow at a constant perfusion pressure did not differ between two groups at baseline or after any Ca\(^{2+}\) stress. At a Ca\(_o\) of 1.0 mM, neither end-diastolic pressure (EDP) nor developed pressure (DP) nor half relaxation time (RT\(_{1/2}\)) during stimulation at 1-3 Hz differed between the two groups; as Ca\(_o\) was increased up to 10 mM, CMH hearts showed a lower threshold for the occurrence of a Ca\(^{2+}\) overload profile: EDP and RT\(_{1/2}\) increased to a greater, and DP to a lesser, extent in CMH than in control hearts. To determine whether calcium influx via Ca\(^{2+}\) channels mediates the lower threshold for Ca\(^{2+}\) overload in CMH hearts, we measured resting pressure and scattered laser light intensity fluctuation (SLIF) in unstimulated hearts. Prior studies have shown that SLIF is generated by microscopic tissue motion caused by diastolic spontaneous sarcoplasmic reticulum Ca\(^{2+}\) release and that SLIF amplitude reflects the extent of cell and sarcoplasmic reticulum Ca\(^{2+}\) loading. The Ca\(^{2+}\)-dependent increase in resting pressure in unstimulated hearts was highly correlated with an increase in SLIF, and this relation was steeper in CMH than in control hearts. CMH hearts also showed a reduced threshold for the occurrence of a Ca\(^{2+}\) overload profile in response to the adrenergic receptor agonists and the Ca\(^{2+}\) channel agonist during electrical stimulation in a Ca\(_o\) of 2.0 mM: maximum DP achieved with each agonist was significantly less and the dose-response curves to each agonist were shifted leftward in CMH versus control hearts. In CMH hearts EDP began to increase at a significantly lower concentration of each agonist, and the maximum extent of increase in EDP in response to all agonists was significantly enhanced compared with control hearts. In response to \(\beta\)-adrenergic or Ca\(^{2+}\) channel agonists, neither resting pressure nor SLIF in unstimulated hearts increased in control or in CMH hearts. In contrast, in response to \(\alpha\)-adrenergic stimulation, both SLIF and resting pressure increased to a greater extent in CMH than in control hearts. These results indicate that in the prehypertrophic CMH heart contractile function is preserved, and no evidence of cellular Ca\(^{2+}\) overload is present during conditions requiring relatively low contractile performance. However, in response to an increase of Ca\(_o\), or to the addition of adrenergic or Ca\(^{2+}\) channel agonists, CMH hearts evidenced a lower threshold for signs of Ca\(^{2+}\) overload than did control hearts. This indicates that this cardiomyopathy is not solely due to vascular pathology and that a latent Ca\(^{2+}\) intolerance of myocardial cells occurs in CMH at an early stage of the disease. That Ca\(^{2+}\) overload could be elicited by an increase in Ca\(_o\) in the absence of electrical stimulation indicates that it is not mediated via Ca\(^{2+}\) influx via Ca\(^{2+}\) channels. (Circulation Research 1991;69:123–133)

The cardiomyopathic Syrian hamster (CMH), BIO 14.6 strain, exhibits a spontaneous hereditary cardiomyopathy that is transmitted as an autosomal recessive trait.\(^1\)-\(^3\) Focal myocytolytic necrosis begins to occur after approximately 30 days of age in females and 10 days later in males; the degenerative changes become most severe about 60-90 days of age in both sexes, and subsequently hypertrophy, fibrosis, and calcification occur.\(^1\)-\(^3\) Additionally, minor morphological abnormalities in males of the BIO 14.6 strain have been noted to occur as early as 30 days of age.\(^4\) It has been demonstrated that the progression of
cardiac damage in these animals is associated with a hyperresponsiveness to catecholamines,5–9 an increased number of Ca2+ channels,10–13 an abnormality in the function of the sarcoplasmic reticulum,14,15 and microvascular spasm–induced ischemia and reperfusion.16–18 Each of these could lead to a common intermediate, that is, an increase in cell and cytosolic Ca2+ that could be related to the focal necrosis occurring at later stages of the disease. Whereas Ca2+ overload has, in fact, been demonstrated at later stages of the disease,19,20 gross abnormalities of Ca2+ homeostasis have not been found at early stages of this disease.20 A decreased cell Ca2+ tolerance during the early stage of the disease, however, might be expected to be “occult,” that is, not associated with gross Ca2+ deposition within the myocardium or its organelles. We hypothesized that even before the cardiac hypertrophic and failure stage, abnormalities of Ca2+ homeostasis in CMH may occur transiently in response to perturbations that increase the contractile state via an increase in cell Ca2+ loading. These abnormalities would lead to the well-recognized functional manifestations of Ca2+ overload, that is, relaxation abnormalities and excessive increases in diastolic pressure, the occurrence of spontaneous Ca2+ oscillations, and limitation of systolic function.21 We therefore studied the response of intact isolated perfused hearts of young (24–45-day-old) male CMH and age- and sex-matched control hamsters to a variety of perturbations that enhance cell Ca2+ loading, including an increase in perfusate [Ca2+] (Ca0), the Ca2+ channel agonist Bay K 8644, and α- or β-adrenergic receptor agonists.

Materials and Methods

Experimental Preparation

Male CMH, BIO 14.6 strain (aged 24–45 days) and age- and sex-matched control hamsters, F1B strain, were purchased from BIO Breeders (Watertown, Mass.) at the age of 2–4 weeks and housed with ordinary diet and water under appropriate conditions until the time of study. Before the hamsters were killed, they were heparinized (1 unit/g body wt) and anesthetized with sodium pentobarbital (0.06 mg/g body wt). The heart was quickly removed, and the aorta was cannulated and retrogradely perfused at a constant pressure (110 cm H2O) at 37°C using well-oxygenated HEPES-buffered solution containing (mM) NaCl 142, KCl 5.0, MgCl2 1.0, HEPES 20.0, glucose 10.0, and CaCl2 2.0, at pH 7.4. To control the heart rate atrioventricular block was effected by mechanical crushing of the atrioventricular nodal region. The hearts were paced with a stimulator (model SD9, Grass Instrument Co., Quincy, Mass.) via bipolar electrodes attached to the right ventricular apex. Pressure was measured with a thin latex balloon, inserted via the left atrium into the left ventricle, connected to a pressure transducer (model P23D, Statham, Oxnard, Calif.), and recorded with a strip-chart recorder (model 220, Gould Inc., Cleveland, Ohio). The pressure signal was A/D converted on-line with a Techmar-Lab Master (Solen, Ohio) acquisition card and an IBM PS/2 computer. End-diastolic pressure (EDP), developed pressure (DP), and time from stimulation to half relaxation (RT1/2) were calculated.

Experimental Procedure

EDP was initially set at 20 mm Hg in all hearts. After a 30-minute equilibration period, EDP fell to 8.2±0.3 and 8.6±0.3 mm Hg in control and CMH hearts, respectively (n=35 for each, p<0.1; NS). In a subset of hearts (n=5 for each group) it was demonstrated that at this level of EDP 86.9±3.9% and 83.7±4.2% of maximum DP occurred in control and CMH hearts, respectively (p<0.1; NS). The volume of the balloon that resulted in the 20 mm Hg EDP was not determined. In another subset of hearts (n=3 for each group) it was shown that after the equilibration period, DP and EDP remained stable for at least 3 hours at a given pacing rate or Ca0. All studies were completed within this time. After equilibration, coronary flow averaged 25.3±0.7 and 23.8±0.8 ml/min/g wet heart wt in control and CMH (n=35 for each), respectively. The effects of the changes in Ca0 were measured over a range of 1.0–10.0 mM at pacing rates of 1, 2, or 3 Hz in the presence of propranolol (5 μM) to block the effect of intrinsic catecholamines released by the electrical stimulation. The response to the Ca2+ channel agonist Bay K 8644 (0.005–1.0 μM) was studied in the presence of 5 μM propranolol in 2.0 mM Ca0 at a pacing rate of 2 Hz. Bay K 8644 was solubilized in polyethylene glycol, which, up to the maximum concentration used with Bay K 8644, had no effect on contraction in control or CMH hearts (n=3 for each). Isoproterenol (0.001–1.0 μM) was used as a β-adrenergic receptor agonist (in the absence of propranolol), and phenylephrine (0.1–10 μM) in the presence of 5 μM propranolol was used as an α-adrenergic receptor agonist. In these experiments the stimulation frequency was 2 Hz and Ca0 was 2.0 mM.

Detection of Spontaneous Ca2+ Oscillations

The occurrence of and changes in the magnitude of microscopic tissue motion caused by spontaneous sarcoplasmatic reticulum Ca2+ release within cardiac cells21 can be determined by measurements of scattered light intensity fluctuation (SLIF) of a laser beam22–25 scattered from the heart.26,27 SLIF was measured as reported previously.26,27 Briefly, a vertically polarized 1-mm-diameter 5 mW He-Ne laser light (λ=632.8 nm) was collimated on the left anterior ventricular surface of perfused hearts at an angle

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TABLE 1. Coronary Flow in Control and Cardiomyopathic Hamster Hearts During Stimulation at 2 Hz

<table>
<thead>
<tr>
<th>Ca\textsubscript{o} response</th>
<th>Control (ml/min/g wet wt)</th>
<th>CMH (ml/min/g wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (2 mM Ca\textsubscript{o})</td>
<td>24.9±1.5 (10)</td>
<td>23.3±1.5 (10)</td>
</tr>
<tr>
<td>10 mM Ca\textsubscript{o}</td>
<td>22.7±1.2 (10)</td>
<td>20.6±1.2 (10)</td>
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<tr>
<td>Bay K 8644 response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (2 mM Ca\textsubscript{o})</td>
<td>25.5±0.7 (10)</td>
<td>24.6±1.3 (10)</td>
</tr>
<tr>
<td>Bay K 8644 (1 μM)</td>
<td>22.5±0.8 (10)</td>
<td>22.0±1.2 (10)</td>
</tr>
<tr>
<td>(\beta)-Adrenergic response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (2 mM Ca\textsubscript{o})</td>
<td>26.5±1.7 (6)</td>
<td>24.4±2.1 (6)</td>
</tr>
<tr>
<td>Isoproterenol (1 μM)</td>
<td>28.6±1.3 (6)</td>
<td>27.1±1.0 (6)</td>
</tr>
<tr>
<td>(\alpha)-Adrenergic response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (2 mM Ca\textsubscript{o})</td>
<td>24.5±1.9 (9)</td>
<td>23.1±1.6 (9)</td>
</tr>
<tr>
<td>Phenylephrine (10 μM)</td>
<td>22.1±1.8 (9)</td>
<td>19.0±1.4 (9)</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Numbers in parentheses indicate number of hearts. No significant difference was found between control and CMH hearts. CMH, cardiomyopathic hamsters; Ca\textsubscript{o}, perfusate [Ca\textsuperscript{2+}].

decreases in cell Ca\textsuperscript{2+} loading.

In the present study SLIF and resting pressure were measured in a subset of hearts in the absence of electrical stimulation in the presence of 5 μM propranolol, which suppressed spontaneous systoles in the ativoventricular-blocked hearts.

**Statistical Analysis**

Data are expressed as mean±SEM and analyzed with unpaired t test or repeated-measures analysis of variance (ANOVA) when appropriate. Statistical significance was taken as p<0.05.

**Materials**

Bay K 8644 was provided by Miles Inc., West Haven, Conn.; phenylephrine and propranolol were purchased from Sigma Chemical Co., St. Louis; and isoproterenol was purchased from Breon Laboratories Inc., New York.

**Results**

**Response to Increases in Ca\textsubscript{o}**

Coronary flow did not vary with Ca\textsubscript{o} and was not different between groups at low or high Ca\textsubscript{o} (Table 1). Figure 1 shows DP and EDP at 1 and 3 Hz stimulation in response to increasing Ca\textsubscript{o} in control and CMH hearts. At a Ca\textsubscript{o} of 1.0 mM, DP and EDP did not differ between the two groups; as Ca\textsubscript{o} was increased, EDP increased to a greater extent and DP to a lesser extent in CMH than in control hearts. Figure 2 shows the relative maximum change in DP with increases in Ca\textsubscript{o} for 1 and 3 Hz stimulation rates. The curves of CMH are significantly leftward of control and the shift is greater with 3 Hz stimulation than 1 Hz stimulation.

Figure 3 depicts the effect of Ca\textsubscript{o} on RT\textsubscript{1/2} at 2 Hz stimulation. In 1.0 mM Ca\textsubscript{o}, no difference was noted between CMH and control hearts. But as Ca\textsubscript{o} increased RT\textsubscript{1/2} became prolonged to a greater extent in CMH than in control hearts; that is, the slope of

**Figure 1.** The developed pressure (DP) and end-diastolic pressure (EDP) in response to increasing perfusate [Ca\textsuperscript{2+}] (Ca\textsubscript{o}) in cardiomyopathic hamster (CMH) and control (C) hearts during stimulation at 1 and 3 Hz. The responses of both DP and EDP are significantly different between control and CMH (p≤0.03, repeated-measures analysis of variance).
the CaO-RT\textsubscript{1/2} relation is markedly steeper in CMH than in control hearts. When relaxation time was calculated as the time from peak pressure to 50% pressure decay, the same pattern emerged. In Ca\textsubscript{0} of 1.0 mM this value was 57.1 ± 1.7 versus 53.5 ± 2.3 msec in control and CMH hearts, respectively (p=NS); at Ca\textsubscript{0} of 10 mM it was 60.3 ± 2.4 versus 73.2 ± 2.1 in control and CMH hearts, respectively (p<0.001).

Figure 4 shows resting pressure (measured in the absence of electrical stimulation) and SLIF across the range of Ca\textsubscript{0} in CMH and control hearts. In 1.0 mM Ca\textsubscript{0} resting pressure did not differ between control and CMH. As Ca\textsubscript{0} increased, resting pressure increased to a greater extent in CMH than in control hearts. In 1.0 mM Ca\textsubscript{0} SLIF was detectable in CMH but was not present in control hearts. The response of SLIF to increases in Ca\textsubscript{0} is significantly steeper in CMH than in control hearts. Note that within each group increases in SLIF and resting pressure are roughly parallel, because under these conditions a change in SLIF reflects a change of Ca\textsuperscript{2+}-dependent resting tone.\textsuperscript{23,25} Taken together, the data in Figures 1–4 indicate that the sensitivity to an increase in Ca\textsubscript{0} is enhanced in CMH versus control hearts.

Response to the Ca\textsuperscript{2+} Channel Agonist Bay K 8644

Coronary flow did not vary between control and CMH during baseline stimulation or in response to Bay K 8644 (Table 1). Figure 5A shows the response of DP and EDP to Bay K 8644 in control and CMH hearts. DP was greater in control (in 2.0 mM Ca\textsubscript{0}) than in CMH hearts in agreement with the Ca\textsubscript{0} dose–response curve in other hearts (Figure 1). In CMH, DP reached maximum and EDP began to increase at lower concentrations of Bay K 8644 than in control hearts. The maximum DP in response to Bay K 8644 was greater in control than in CMH hearts (Table 2). Figure 5B shows the data in Figure 5A expressed relative to the maximum change of DP in each heart. The curve for CMH hearts is significantly shifted leftward of that of control hearts: the concentration of Bay K 8644 to achieve the half-maximal response in DP was 0.017 ± 0.002 \(\mu\text{M}\) in CMH and 0.034 ± 0.004 \(\mu\text{M}\) in control hearts (n=10 for each; p<0.01). In the presence of Bay K 8644, RT\textsubscript{1/2} became more prolonged in CMH than in control hearts (Figure 6), as was the case when Ca\textsubscript{0} was increased (Figure 3). Relaxation time calculated as time from peak pressure to half pressure decay was 55 ± 1.5 and 53.7 ± 2.5 msec in control and CMH.
hearts, respectively; after 1 μM Bay K 8644 it increased to 60±1.4 and to 73.7±2.8 msec in control and CMH hearts, respectively (p<0.001). Thus, CMH hearts showed significantly enhanced sensitivity to the Ca2+ channel agonist. In the absence of pacing, neither resting pressure nor SLIF increased with Bay K 8644 in control or in CMH hearts. This is expected because the increase in cell Ca2+ loading caused by Bay K 8644 occurs only during stimulation and dissipates after the termination of the electrical stimulation, as Ca2+ channels become inactivated but Ca2+ efflux from the cell continues to occur.25

Response to β-Adrenergic Receptor Stimulation

Figure 7 shows the response of DP and EDP to isoproterenol in CMH and control hearts. DP before drug (Ca0=2.0 mM) and maximum DP achieved with isoproterenol were significantly greater in control than in CMH hearts (Table 2). However, in CMH hearts the maximum DP occurred at a significantly lower concentration of isoproterenol compared with control. The curve of CMH hearts is shifted significantly leftward from that of control hearts: the concentration of isoproterenol to achieve the half-maximal response in DP was 0.010±0.001 μM in CMH and 0.019±0.001 μM in control hearts (n=6 for each; p<0.01). Thus, CMH myocardium shows higher sensitivity to the β-adrenergic receptor agonist isoproterenol compared with control hearts. EDP decreased with isoproterenol in control hearts; a small decrease in EDP at lower concentrations also occurred in CMH hearts but, in contrast to control hearts, at higher isoproterenol concentrations EDP increased in CMH hearts. Before isoproterenol (in

![Figure 4. Resting pressure (RP) and scattered laser light intensity fluctuation (SLIF) measured across the range of perfusate [Ca2+] (Ca0) in cardiomyopathic hamster (CMH) and control (C) hearts. The responses of both RP and SLIF are significantly different between control and CMH (both p<0.01, repeated-measures analysis of variance).](image)

![Figure 5. Panel A: The response of developed pressure (DP) and end-diastolic pressure (EDP) to Bay K 8644 (perfusate [Ca2+] =2.0 mM) in control (C) and cardiomyopathic hamster (CMH) hearts. The responses of both DP and EDP are significantly different between control and CMH (p<0.01 and p<0.02 for DP and EDP, respectively, repeated-measures analysis of variance). Panel B: The DP data in CMH and control in panel A expressed relative to the maximum change of DP in each heart. The curves are significantly different between control and CMH (p<0.01, repeated-measures analysis of variance).](image)
the absence of propranolol) was administered, baseline RT1/2 was greater in CMH than in control hearts, and with isoproterenol it shortened in both control and CMH hearts in a concentration-dependent manner (Figure 8A). However, the relative response was attenuated in CMH compared with control hearts (Figure 8B). Relaxation time measured as the time from peak pressure to 50% pressure decay was 42.9±1.6 and 50.3±2.5 msec before isoproterenol in control and CMH hearts, respectively (p<0.01); in the presence of 1 μM isoproterenol this value was 41.1±1.4 versus 55.2±4.3 msec in control and CMH hearts, respectively (p<0.001). Coronary flow did not differ between groups (Table 1). SLIF could not be measured in the absence of electrical stimulation in the presence of isoproterenol because of the spontaneous systoles and extrasystoles that occurred when stimulation was stopped in the absence of propranolol. But, as in the case with Bay K 8644, after cessation of stimulation in the presence of isoproterenol, the gain in cell Ca2+ achieved during stimulation dissipates, and no change in either SLIF or resting pressure would be expected to occur.25

Response to α-Adrenergic Receptor Stimulation

Figure 9A shows the response of DP and EDP to phenylephrine plus 5 μM propranolol in control and CMH hearts. The maximum DP achieved with phenylephrine was greater in control than in CMH hearts (Table 2). Figure 9B shows the DP data of Figure 9A expressed relative to the maximum change in DP in each heart. The curve of CMH is significantly shifted leftward that of control hearts: the concentration of phenylephrine required to achieve the half-maximal response in DP was 0.21±0.03 μM in CMH and 0.48±0.08 μM in control hearts (n=9 for each; p<0.01). At higher concentrations of phenylephrine, EDP increased in CMH but did not increase in control hearts. RT1/2 increased to a greater extent in CMH than in control hearts (Figure 10). Relaxation time measured from peak pressure to 50% pressure decay was 53.7±2.2 and 59.9±2.7 msec in control and CMH hearts, respectively (p<0.02). In the presence of 10 μM phenylephrine, this value was 60.3±2.3 and 70.5±4.5 msec in control and CMH hearts, respectively (p<0.01). Figure 11 shows the response of resting pressure and SLIF to phenylephrine. Before application of the drug, resting pressure did not differ between groups; SLIF was undetectable in control but was

![Figure 7](image_url)  **FIGURE 7.**  The response of developed pressure (DP) and end-diastolic pressure (EDP) to isoproterenol in cardiomyopathic hamster (CMH) and control (C) hearts. The responses of both DP and EDP are significantly different between control and CMH hearts (p<0.01 and p<0.02 for DP and EDP, respectively, repeated-measures analysis of variance).

![Figure 6](image_url)  **FIGURE 6.**  The effect of Bay K 8644 on the time from stimulation to half relaxation (RT 1/2) in cardiomyopathic hamster (CMH) and control hearts. The response is significantly different between control and CMH hearts (p<0.01, repeated-measures analysis of variance).

### Table 2. Baseline and Maximum Developed Pressure

<table>
<thead>
<tr>
<th></th>
<th>Control (mm Hg)</th>
<th>CMH (mm Hg)</th>
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<tbody>
<tr>
<td>Ca_o response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (2 mM Ca_o)</td>
<td>86.1±2.3 (10)</td>
<td>67.1±2.0 (10)*</td>
</tr>
<tr>
<td>Maximum DP</td>
<td>178.4±2.5 (10)</td>
<td>133.4±1.9 (10)*</td>
</tr>
<tr>
<td>Bay K 8644 response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (2 mM Ca_o)</td>
<td>96.4±4.0 (10)</td>
<td>63.9±1.9 (10)*</td>
</tr>
<tr>
<td>Maximum DP</td>
<td>193.3±2.7 (10)</td>
<td>137.1±2.0 (10)*</td>
</tr>
<tr>
<td>β-Adrenergic response</td>
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<tr>
<td>Baseline (2 mM Ca_o)</td>
<td>92.0±6.2 (6)</td>
<td>63.6±2.0 (6)*</td>
</tr>
<tr>
<td>Maximum DP</td>
<td>194.5±3.1 (6)</td>
<td>154.7±2.4 (6)*</td>
</tr>
<tr>
<td>α-Adrenergic response</td>
<td></td>
<td></td>
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<tr>
<td>Baseline (2 mM Ca_o)</td>
<td>98.9±3.8 (9)</td>
<td>62.1±2.4 (9)*</td>
</tr>
<tr>
<td>Maximum DP</td>
<td>159.2±2.3 (9)</td>
<td>135.5±2.7 (9)*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Numbers in parentheses indicate number of hearts. CMH, cardiomyopathic hamsters; Ca_o, perfusate [Ca2+]; DP, developed pressure. *p<0.01, CMH vs. control.
present in CMH. The phenylephrine responses of both resting pressure and SLIF were significantly steeper in CMH than in control hearts. These responses were completely abolished in the presence of an α-adrenergic receptor antagonist, 1.0 μM prazosin (not shown). Coronary flow did not differ between groups in response to phenylephrine (Table 1).

**Discussion**

The present results show that in isolated hearts from the 24–45-day-old CMH perfused with 1.0 mM Ca\textsubscript{0}, neither DP, EDP, nor RT\textsubscript{1/2} in the presence of propranolol differed from those of control hearts. As Ca\textsubscript{0} was increased, DP increased to a lesser and EDP to a greater extent in CMH than in control hearts, and RT\textsubscript{1/2} in CMH hearts became prolonged. In a prior study the developed force production of isolated papillary muscles of hypertrophic 120-day-old CMH hearts bathed in 2.4 mM Ca\textsubscript{0} also exhibited a reduction of developed tension generation and prolonged relaxation.\textsuperscript{29} In the present study, not only was the maximum DP achieved in response to an increase in Ca\textsubscript{0} less in CMH hearts, but it also occurred at a lower Ca\textsubscript{0}; the dose–response curve was shifted leftward. In unstimulated hearts a greater Ca\textsubscript{0}-dependent rise in EDP and resting pressure occurred in CMH than in control hearts. The Ca\textsubscript{0}-induced increase in SLIF, a monitor of spontaneous,

**Figure 8.** Panel A: The effect of isoproterenol on the time from stimulation to half relaxation (RT \textsubscript{1/2}) in cardiomyopathic hamster (CMH) and control hearts. The response is significantly different between control and CMH hearts (p≤0.01, repeated-measures analysis of variance). Panel B: The relative change of RT \textsubscript{1/2} in response to isoproterenol. The response is significantly different between control and CMH (p≤0.02, repeated-measures analysis of variance).

**Figure 9.** Panel A: The response in developed pressure (DP) and end-diastolic pressure (EDP) in control (C) and cardiomyopathic hamster (CMH) hearts to phenylephrine. Both DP and EDP responses are significantly different between control and CMH (both p≤0.01, repeated-measures analysis of variance). Panel B: The data of panel A expressed relative to the maximum DP in each heart. Both curves are significantly different between control and CMH (p≤0.01, repeated-measures analysis of variance).
heterogeneous sarcoplasmic reticulum–myofilament Ca\textsuperscript{2+} cycling\textsuperscript{21–27} was greater in CMH than in control hearts. This profile in CMH hearts (i.e., a leftward shift of the response in DP, a reduction in maximum DP, relaxation abnormality, and excessive increases in EDP, resting pressure, and SLIF\textsuperscript{2}) is characteristic of the Ca\textsuperscript{2+} overload state\textsuperscript{21,23,26,27} and indicates that, as Cao\textsubscript{0} was raised, cell Ca\textsuperscript{2+} loading increased to a greater extent in CMH than in control hearts.

In response to perturbations that increase cell Ca\textsuperscript{2+} loading at a constant Cao\textsubscript{0}, that is, α- and β-adrenergic agonists and the dihydropyridine Ca\textsuperscript{2+} channel agonist Bay K 8644, this Ca\textsuperscript{2+} overload profile also occurred to a greater extent in CMH than in control hearts. The Ca\textsuperscript{2+} overload in response to the β-adrenergic agonist and Bay K 8644, unlike that in response to an increase in Cao\textsubscript{0} and α-adrenergic agonist, occurs only during stimulation and dissipates after a rest period.\textsuperscript{23,24} In contrast, after the termination of the electrical stimulation in the presence of 10 μM phenylephrine, both SLIF and resting pressure were significantly increased in CMH hearts, whereas neither SLIF nor resting pressure increased in control hearts. Note, however, that in contrast to a similar result after an increase in Cao\textsubscript{0}, this result does not directly indicate that the actual amplitude of cytosolic Ca\textsuperscript{2+} oscillations in the absence of stimulation is elevated to a greater extent in CMH than in control hearts in the presence of 10 μM phenylephrine, since α-adrenergic stimulation leads to an increase in the myofilament responsiveness to Ca\textsuperscript{2+}.\textsuperscript{30,31} However, it has recently been demonstrated that 10 μM phenylephrine elevated cytosolic Ca\textsuperscript{2+} in the heart cells separated from 30-day-old CMH, whereas it did not do so in cells from age-matched control hearts.\textsuperscript{32} Also, a previous study in papillary muscles of 30-day-old CMH showed a greater sensitivity of developed force to an α-adrenergic agonist.\textsuperscript{7}

The present results interpreted collectively suggest that, while under basal conditions, that is, during stimulation at 1 Hz in 1.0 mM Cao\textsubscript{0} in the presence of propranolol, there is no evidence for cell Ca\textsuperscript{2+} overload in nonnecrotic CMH myocardial tissue,\textsuperscript{30} the net cell Ca\textsuperscript{2+} gain that occurs with the ionic or pharmacological perturbations used is greater in CMH than in control hearts. This would indicate that either enhanced Ca\textsuperscript{2+} influx or a reduced rate of Ca\textsuperscript{2+} efflux occurs in CMH versus control hearts in response to these Ca\textsuperscript{2+} loading perturbations. A greater reduction of global coronary flow in CMH than in control hearts cannot explain the present results because coronary flow did not differ between CMH and control hearts, either at baseline conditions or during the Ca\textsuperscript{2+} loading protocols (Table 1). Additionally, if microcirculatory failure were a cause of the Ca\textsuperscript{2+} overload in CMH hearts, SLIF would be expected to decrease under the relative ischemic condition, because SLIF is suppressed during ischemia.\textsuperscript{26,27} Additionally, vascular mechanisms for myocyte overload in response to Ca\textsuperscript{2+} stresses can be avoided in studies of single cardiac myocytes. Recently, it has been shown that single cardiac myocytes isolated from 30-day-old CMH hearts show evidence of greater Ca\textsuperscript{2+} loading during an increase in Cao\textsubscript{0} than do cells in control hearts.\textsuperscript{33} At 8 months of age, as cellular hypertrophy develops, the time-averaged calcium concentration of CMH cells is greater than in control hearts.\textsuperscript{34}

Multiple nonvascular mechanisms could be implicated in the greater relative Ca\textsuperscript{2+} overload in the CMH versus control myocardium provoked by the various perturbations in the present study. An in-

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**Figure 10.** The effect of phentolamine on the time from stimulation to half relaxation (RT 1/2) in cardiomyopathic hamster (CMH) and control hearts. The response is significantly different between control and CMH (p<0.01, repeated-measures analysis of variance).

**Figure 11.** The responses of resting pressure (RP) and scattered laser light intensity fluctuation (SLIF) to phentolamine in cardiomyopathic hamster (CMH) and control (C) hearts at 2.0 mM perfusate [Ca\textsuperscript{2+}]. Both RP and SLIF responses are significantly different between control and CMH (p<0.01, repeated-measures analysis of variance).
crease in cardiac Ca$^{2+}$ channel density (based on specific $[^{3}H]$nitrendipine binding sites) has been reported to occur in 25-day-old CMH. An increase in each of Ca, $\beta$-adrenergic stimulation, and Bay K 8644 could cause enhanced Ca$^{2+}$ influx via an enhanced Ca$^{2+}$ channel density. We do not favor this as an exclusive hypothesis, however, because even in the absence of electrical stimulation, that is, at the resting membrane potential when "L-type" Ca$^{2+}$ channels are not activated, an increase in Ca, caused an excessive increase in resting pressure and SLIF in CMH versus control hearts (Figure 4). Additionally, increasing Ca, in the presence of a high concentration of a calcium channel blocker (25 $\mu$M verapamil) caused a similarly greater augmentation of resting pressure and SLIF in CMH versus control hearts in the absence of stimulation as in the present study (O. Hano, unpublished data). In unstimulated rat cardiac muscle, the gain in intracellular Ca$^{2+}$ after increases in Ca, has been linked to the extent to which intracellular Na$^{+}$ is augmented. Thus, an increase in cytosolic sodium concentration in CMH versus control hearts could explain a greater increase in Ca, in CMH versus control hearts after an increase in Ca,.

Changes in the density or affinity of $\beta$- and $\alpha$-adrenergic receptors in CMH hearts could possibly be implicated in the Ca$^{2+}$ overload observed in response to $\alpha$- and $\beta$-agonists in CMH hearts in the present study. However, the density of $\alpha$- and $\beta$-adrenergic receptors in CMH examined by $[^{3}H]$prazosin and $[^{3}H]$pindolol binding is not altered in 21-day-old CMH and is only slightly increased at 35 days in CMH compared with age-matched controls. Also, more recent studies suggest a defect in the stimulatory G proteins coupling the adrenergic receptor to second-messenger mechanisms at 27 days in CMH. Thus, as in the case for the Ca$^{2+}$ channel receptor hypothesis, increased density of catecholamine receptors is not sufficient to explain the enhanced Ca$^{2+}$ overload in response to either $\alpha$- or $\beta$-adrenergic receptor agonists.

While multiple specific subcellular mechanisms that govern Ca$^{2+}$ influx, for example, altered Ca$^{2+}$ channels, or $\alpha$- and $\beta$-adrenergic signal transduction pathways might have some role in the observed results, a net decrease in cell Ca$^{2+}$ buffering capacity or in Ca$^{2+}$ efflux in response to the perturbations used in the present study is also a plausible explanation for the observed results. Possible mechanisms include a reduction in sarcolemmal Ca$^{2+}$ pump activity, altered Na$^{+}$-Ca$^{2+}$ exchange, a reduction in the Na$^{+}$-$K^{+}$ pump activity, and a diminution of Ca$^{2+}$ uptake or enhanced spontaneous efflux by the sarcoplasmic reticulum. Regarding biochemical abnormalities in the sarcosomal membrane of CMH hearts, it has been reported that while ATP-independent Ca$^{2+}$ binding initially increases at 55 days of age, ATP-dependent Ca$^{2+}$ binding capacity decreases at 90 days of age. Ca$^{2+}$-dependent ATPase decreases only in the late stage of the disease in the CMH, UMX7.1 strain, which is derived from the BIO 14.6 strain. However, a reduction of Ca$^{2+}$-ATPase of cardiac sarcolemma at 40 days of age in the BIO 53.58 strain has been reported in another study. Similar interstudy differences have been observed in the case of Na$^{+}$-Ca$^{2+}$ exchange. In one study, while no alteration was observed at 40 days of age in CMH hearts, Na$^{+}$-dependent Ca$^{2+}$ uptake decreased at 120 and 240 days of age in the UMX7.1 strain versus control hearts. More recently it has been observed that Na$^{+}$-Ca$^{2+}$ exchange in Na$^{+}$-loaded sarcolemmal vesicles is enhanced at 30 days in the CMH, BIO 14.6 strain. These different results might, in part, be due to the strain studied, for example, due to the different severity of disease between strains. No change in Ca$^{2+}$ sequestration by isolated sarcoplasmic reticulum occurs in the early phase of the disease. However, during later stages of the disease, that is, at 9 months of age, the initial rate and capacity of Ca$^{2+}$ uptake by the sarcoplasmic reticulum and Ca$^{2+}$-ATPase activities from the sarcoplasmic reticulum vesicles become significantly decreased. Regarding Na$^{+}$,K$^{+}$-ATPase activity, while the cytosolic Na$^{+}$ concentration has not been measured in CMH hearts during the prenecrotic stage, a reduced Na$^{+}$,K$^{+}$-ATPase activity under certain experimental conditions has been observed as early as 25 days in UMX7.1 in the CMH strain. However in the BIO 53.58 strain no significant changes in Na$^{+}$,K$^{+}$-ATPase activity were detected at 40 days of age.

In summary, based on the current literature, specific abnormalities in none of the cell surface or intracellular biochemical mechanisms that regulate the cell ionic homeostasis can be firmly linked to the abnormalities of Ca$^{2+}$ homeostasis demonstrated in the prehypertrophic CMH hearts investigated in the present study. Thus, additional and more focused measurements of mechanisms of ionic control are required in this regard.

Regardless of the specific cellular mechanism involved, the tendency for young CMH hearts to become Ca$^{2+}$ overloaded in response to a Ca$^{2+}$ stress, as observed in the present study, need not be homogeneous among myocardial cells and could progressively increase with age. This could eventually lead to a focal Ca$^{2+}$ necrosis observed at later stages in the disease. Additionally, an abnormal Ca$^{2+}$ loading by vascular smooth muscle cells in vivo, leading to spasm after ischemia and reperfusion, could also contribute to altered myocyte cell Ca$^{2+}$ handling and be implicated in focal necrosis at later stages of the disease. However, the present results, in providing evidence for a myocardial cell component, suggest that the Ca$^{2+}$ overload of the cardiomyopathy that later develops is not solely vascular in origin.

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