Cardiomyocyte function associated with hyperactivity and/or hypertension in genetic models of LV hypertrophy

Bradley M. Palmer,1 Zengyi Chen,2 Richard R. Lachapelle,2 Edith D. Hendley,1 and Martin M. LeWinter1,2

1Department of Molecular Physiology and Biophysics, 2Department of Medicine, University of Vermont, Burlington, Vermont 05405

Submitted 29 March 2005; accepted in final form 8 September 2005

Cardiomyocyte function associated with hyperactivity and/or hypertension in genetic models of LV hypertrophy. Am J Physiol Heart Circ Physiol 290: H463–H473, 2006. First published October 21, 2005; doi:10.1152/ajpheart.00310.2005.—We examined cardiomyocyte intracellular calcium ([Ca2+]i) dynamics and sarcomere shortening dynamics in genetic rat models of left ventricular (LV) hypertrophy associated with or without hypertension (HT) and with or without hyperactive (HA) behavior. Previous selective breeding of the spontaneously hypertensive rat (SHR) strain, which is HA and HT, with the Wistar-Kyoto (WKY) rat strain, which is not hyperactive (NA) and not hypertensive (NT), has led to two unique strains: the WKHA strain, selected for HA and NT, and the WKHT strain, selected for NA and HT. Cardiomyocytes were isolated from young adult males and females of each strain, paced at 2, 3, and 4 Hz in 1.2 mM external Ca2+ concentration at 37°C, and cardiomyocyte [Ca2+]i, and sarcomere dynamics were recorded simultaneously. Under these conditions, LV cardiomyocyte systolic and diastolic [Ca2+]i dynamics and diastolic sarcomere dynamics in the WKHT were significantly enhanced compared with WKY controls, suggesting an underlying LV hypertrophic response that successfully compensated for HT in the absence of HA. LV cardiomyocyte [Ca2+]i dynamics in the WKHA and SHR were strikingly similar to each other and only slightly reduced compared with WKY. LV cardiomyocyte systolic and diastolic sarcomere dynamics, on the other hand, were significantly reduced in the SHR compared with WKHA and more so in male than in female SHR. We conclude from these data that HT alone is an insufficient descriptor of the cause of LV hypertrophy and diminished LV cardiomyocyte function in the SHR rat. These data further suggest that HA (augmented by male sex) in the SHR may interact with the HT state to initiate impaired cardiomyocyte function and thereby inhibit or undermine an otherwise compensatory response that may occur with HT in the absence of HA.

spontaneously hypertensive rat; Wistar-Kyoto rat; Wistar-Kyoto hypertensive; Wistar-Kyoto hyperactive; fura-2; left ventricular

The spontaneously hypertensive rat (SHR) has served for decades as one of the preferred models for studies of LV hypertrophy and associated dysfunction (2). Although severe hypertension (HT) is the cardiovascular attribute most commonly associated with the SHR strain, the SHR is also behaviorally hyperactive (HA) and hyperreactive to stress (26). These latter traits are not usually seen in other models of genetic or experimental hypertension (26), and have been fixed fortuitously by cosegregation during inbreeding of the SHR. As early as 1977, it was suggested that LV hypertrophy and dysfunction in the SHR may not be solely due to HT and may be related to some degree to HA or other traits (9, 10). Direct evidence for more than one basis for LV hypertrophy was first reported in the SHR, which was rendered normotensive by means of peripheral sympathectomy using nerve growth factor antisemur or hydralazine and yet developed LV hypertrophy and dysfunction (1, 9, 10, 34).

Genetic distinctions between the HA and HT traits have been demonstrated using a recombinant inbreeding program initiated by crossbreeding the SHR with its control Wistar-Kyoto (WKY) strain, which is not hyperactive (NA) and not hypertensive (NT) (16). The HA and HT phenotypes are now segregated into two homozygous strains: the WKHA, which is HA and NT, and the WKHT, which is NA and HT (19, 20). Interestingly, the WKHA strain develops LV hypertrophy comparable to that of the conventional SHR by mechanisms clearly independent of HT (12, 20). Although not yet tested, the mechanisms underlying LV hypertrophy in the WKHA may include HA-related elevated sympathetic activity, both systemic (9, 17, 24) and localized to cardiac tissue (1, 33), which was found in the SHR with LV hypertrophy independent of HT (9, 10).

The WKHT strain likewise develops significant LV hypertrophy, which is independent of HA or elevated sympathetic activity in cardiac tissue (18, 19). Stellate ganglion cells and vascular smooth muscle, however, are hypertrophied leading to elevated peripheral vascular resistance in the WKHT and SHR but not in the WKHA and WKY strains (31). The heightened peripheral vascular resistance in the two HT strains is also accompanied by hyperinnervation of nerves containing neuropeptide Y (15), decreased expression of anterior pituitary proopiomelanocortin (4), and a mutation in the nerve growth factor receptor gene (28), all of which ultimately promote or facilitate growth of sympathetic nerves and smooth muscle in the peripheral vasculature. The WKHT strain is therefore characterized as HT due to high peripheral vascular resistance without the accompanying factors of HA and hyperreactivity to stress found in the SHR.

Studies comparing WKHA and WKHT strains with the progenitor SHR and WKY strains have previously revealed that hyperreactivity of behavioral and cardiovascular responses to stressful stimuli is a characteristic that appears to have cosegregated with the HA trait and not the HT trait (7, 17, 23). Specifically relevant to cardiovascular responses, for example,
circuiting norepinephrine and epinephrine in response to foot-shock rise more dramatically in the hyperactive SHR and WKHA strains than in the nonhyperactive WKY and WKHT, regardless of the presence of HT (8, 17, 21). Similarly, elevation of mean arterial pressure and reduction of hindquarter vascular resistance occurring after an air-jet stimulus were significantly augmented by the HA trait independent of HT (23). It appears likely that cosegregation of HT with hyperreactivity to stress in the SHR has occurred while developing the SHR from the parental WKY rat, considering that stressful conditions (restraint and heat) accompanied the measurement of systolic pressure, an elevation of which was the criterion used for selection (36).

Although we will refer to LV hypertrophy and cardiomyocyte function in the WKHA, WKHT, and SHR as being associated with HA alone, HT alone, and the combination of HA and HT, respectively, it is very important to note that the LV hypertrophic responses in these strains may be partly or even entirely initiated by other traits not yet identified or characterized in these strains. Nevertheless, it is clear that HT does not contribute to LV hypertrophy in the WKHA, and that HA and hyperreactivity to stress do not contribute to LV hypertrophy in the WKHT. With this knowledge in hand, we hypothesized that LV cardiomyocyte function would be differentiated among these rat models of LV hypertrophy associated with the presence and/or absence of HA and HT. We found that LV cardiomyocyte intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) and shortening dynamics in the WKHT were significantly enhanced, implying that the myriad of possible triggers initiating LV hypertrophy in the WKHT, possibly including HT and certainly excluding HA, lead to significantly enhanced LV cardiomyocyte function. In contrast, LV cardiomyocyte function was significantly diminished in the SHR and more so in male SHR. These data collectively imply that additional mechanisms besides HT, possibly HA and certainly male sex, in the SHR constitute important, if not necessary, contexts within which to interpret LV cardiomyocyte dysfunction in the SHR.

**METHODS**

**Animals**

Male (M) and female (F) rats of four inbred (fully homozygous) strains, all derived originally from WKY rats, were subjects of this study. WKHA (F45-F46 generations) and WKHT (F42-F45 generations) were from the colony maintained at the University of Vermont since 1980 (19). SHR and WKY were obtained commercially from Harlan Sprague Dawley (Indianapolis, IN).

Rats were housed in a 12-h/12-h light-dark cycle and given standard rat chow and water ad libitum. At 9–12 wk of age, the hearts and lungs from four rats in each group were excised, bronchials and right ventricle removed, and wet weights of remaining parenchyma and LV recorded. The LV was clamp frozen with liquid N\(_2\) and stored at −80°C for later detection of calcium regulatory proteins sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA2a) and phospholamban (PHB). Hearts from six rats in each group underwent LV cardiomyocyte isolation. The latter procedure precluded analysis of LV mass. Animal care and use were conducted under the guidelines accepted by the American Physiological Society and received prior approval from the Institutional Animal Care and Use Committee at the University of Vermont.

**Activity, Blood Pressure, and Heart Rate Measurements**

Behavioral and cardiovascular characteristics of the four strains are presented in Table 1. These data were obtained from more than 500 rats inbred at the University of Vermont over the last 10 years. Behavioral and cardiovascular measurements were carried out in rats at 11–14 wk of age. Spontaneous locomotor activity was measured by using a 30-cm × 30-cm square translucent cage fitted with matching light beams and detectors, which permitted counting of the number of beam interruptions in a 15-min period (19). Arterial blood pressure and heart rate were determined by noninvasive tail plethysmography by using a computerized blood pressure monitor (model 31, ITLC/Life Science Instruments, Woodland Hills, CA). Rats were restrained in a lucite holder set in a chamber at 33–35°C until they settled quietly (at least 15 min) before three to five tail-cuff inflations were carried out for blood pressure and heart rate determination.

**Western Blot Analysis of SERCA2a and PHB Content**

Left ventricles that had been clamp frozen in liquid N\(_2\) were homogenized in SDS-containing buffer. Total protein content of homogenates was determined by Bradford assay, and equal LV

**Table 1. Characteristics of inbred strains from the University of Vermont colony, 1996 to present**

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>WKHT</td>
<td>WKHA</td>
<td>SHR</td>
</tr>
<tr>
<td>Number of rats</td>
<td>54</td>
<td>80</td>
<td>93</td>
<td>39</td>
</tr>
<tr>
<td>Activity, counts/min</td>
<td>257 ± 13 (^{a,b,c,d})</td>
<td>343 ± 15 (^{a,c,d})</td>
<td>572 ± 17 (^b)</td>
<td>586 ± 22 (^b)</td>
</tr>
<tr>
<td>Mean pressure, mmHg</td>
<td>107 ± 2 (^{b,d})</td>
<td>140 ± 2 (^{a,c,d})</td>
<td>110 ± 1 (^{b,d})</td>
<td>149 ± 2 (^{a,c,d})</td>
</tr>
<tr>
<td>Diastolic pressure, mmHg</td>
<td>88 ± 1 (^{c,d})</td>
<td>125 ± 2 (^{a,c,d})</td>
<td>96 ± 1 (^{b,d})</td>
<td>132 ± 2 (^{a,c,d})</td>
</tr>
<tr>
<td>Systolic pressure, mmHg</td>
<td>122 ± 2 (^{a,c,d})</td>
<td>170 ± 2 (^{a,c,d})</td>
<td>138 ± 3 (^{b,d})</td>
<td>183 ± 3 (^{a,c,d})</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>323 ± 5 (^{c,d})</td>
<td>319 ± 4 (^{d})</td>
<td>353 ± 3 (^{b})</td>
<td>342 ± 5 (^{b})</td>
</tr>
<tr>
<td>Body mass, g</td>
<td>309 ± 3 (^{b,d})</td>
<td>318 ± 3 (^{a,c,d})</td>
<td>292 ± 3 (^{b})</td>
<td>297 ± 3 (^{b})</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of rats</td>
<td>52</td>
<td>119</td>
<td>102</td>
<td>46</td>
</tr>
<tr>
<td>Activity, counts/min</td>
<td>279 ± 10 (^{a,c,d})</td>
<td>332 ± 10 (^{c,d})</td>
<td>602 ± 13 (^{b})</td>
<td>635 ± 19 (^{b})</td>
</tr>
<tr>
<td>Mean pressure, mmHg</td>
<td>96 ± 2 (^{b,c,d})</td>
<td>126 ± 1 (^{a,c,d})</td>
<td>105 ± 1 (^{b,d})</td>
<td>134 ± 7 (^{a,c,d})</td>
</tr>
<tr>
<td>Diastolic pressure, mmHg</td>
<td>86 ± 2 (^{a,c,d})</td>
<td>114 ± 1 (^{a,c,d})</td>
<td>93 ± 1 (^{b,d})</td>
<td>121 ± 2 (^{a,c,d})</td>
</tr>
<tr>
<td>Systolic pressure, mmHg</td>
<td>115 ± 2 (^{a,c,d})</td>
<td>150 ± 1 (^{a,c,d})</td>
<td>128 ± 1 (^{b,d})</td>
<td>160 ± 2 (^{a,c,d})</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>374 ± 5 (^{b,a})</td>
<td>351 ± 3 (^{a,c})</td>
<td>420 ± 3 (^{b})</td>
<td>374 ± 10 (^{a})</td>
</tr>
<tr>
<td>Body mass, g</td>
<td>210 ± 2 (^{a,b,c,d})</td>
<td>197 ± 1 (^{a,c})</td>
<td>185 ± 1 (^{b,d})</td>
<td>189 ± 2 (^{a,c,e})</td>
</tr>
</tbody>
</table>

Values are means ± SE. WKY, Wistar-Kyoto rats; WKHT, WKY hypertensive; non hyperactive rats; WKHA, WKY hypertensive; not hypertensive rats; SHR, spontaneously hypertensive, hyperactive rats. Post hoc differences are indicated by the following: *different from WKY; †different from WKHT; ‡different from WKHA; §different from SHR of same sex at \(P < 0.05\) level. ¶Different from males of the same strain at \(P < 0.05\) level.

**AJP-Heart Circ Physiol.** VOL 290 • JANUARY 2006 • www.ajpheart.org
proteins were loaded for each population were run on electrophoresis gels and transferred to nitrocellulose using standard methods. Primary antibodies for actin, SERCA2a, and PHB and secondary hors eradish peroxidase goat, anti-mouse antibody (Chemicon, Temecula, CA) were used to detect protein content by standard chemiluminescence. The detected content of SERCA2a and PHB was normalized to detected content of actin from the same electrophoresis and transfer procedures.

Left Ventricular Cardiomyocyte Isolation

Left ventricular cardiomyocytes were obtained from the LV septal and free wall by using methods described previously (29). All chemicals and reagents were obtained from Sigma (St. Louis, MO) except where noted. In brief, animals were administered heparin (500 U ip) and then anesthetized with pentobarbital sodium (90 mg/kg ip) (Abbott Laboratories, North Chicago, IL). Hearts were rapidly excised and placed in ice-cold saline. The aorta was then cannulated, and the heart coronary bed was perfused by using a modified Langendorf perfusion apparatus, which delivered a bicarbonate-based Krebs-Henseleit buffer with nominal free Ca2+(in mM: 113 NaCl, 4.7 KCl, 0.6 KH2PO4, 1.2 MgSO4, 12 NaHCO3, 12 KHCO3, 20 glucose, 10 HEPES, 30 taurine, 2 carnitine, 2 creatine, and 30 2.5-butanediole monoamine (BDM) plus 1% MEM amino acid) and a solution containing collagenase 196 U/ml (Worthington, Freehold, NJ) and trypsin 0.25 mg/ml. All solutions were maintained at pH 7.4 and 37°C and bubbled with 95% O2-5% CO2 gas.

After the heart was palpably flaccid (between 20 and 30 min), the atria and right ventricular free wall were removed. The left ventricular free wall and septum were then minced and placed in the collagenase and trypsin solution. Isolated cardiomyocytes were suspended in bicarbonate-base buffer, filtered through a nylon mesh to remove free wall and septum were then minced and placed in the collagenase and trypsin solution. Isolated cardiomyocytes were suspended in bicarbonate-base buffer, filtered through a nylon mesh to remove fibrous tissue and debris, washed three times by centrifugation for 5 min at 100 g, resuspended in 15 mM BDM bicarbonate buffer, and gradually exposed to increasing Ca2+ concentrations from 0.05, 0.25, 0.5, to 1.2 mM. Cardiomyocytes were kept in 1.2 mM Ca2+ loading buffer (137 NaCl, 5.4 KCl, 0.5 MgCl2, 10 HEPES, 5.5 glucose, and 0.5 Probenecid) until use.

Experimental Protocol

Cardiomyocytes were dropped onto a heated flow-through chamber (Warner Instruments, Hamden, CT), which was placed on the stage of an inverted microscope (Nikon Diaphot) fitted with a ×40 objective. Superfusion of the loading solution was maintained at 37°C. Cardiomyocytes were electrically paced via field stimulation by using platinum electrodes with stimulus duration of 0.5 ms and voltage of 1.5 times their threshold of stimulation (Grass Instruments, Boston, MA). Sarcosarcomere shortening dynamics were recorded for four cardiomyocytes per heart, and [Ca2+]i dynamics were recorded for four cardiomyocytes per heart after cardiomyocytes had achieved steady-state contractile function at each stimulation frequency of 2, 3, and 4 Hz. The number of cardiomyocytes used to characterize a single heart was calculated as that number that would guarantee the standard error of the mean for intraheart variability to be less than half of the interheart variability. For example, the standard deviation of cardiomyocyte peak shortening within one heart was found to be 2.2%; for four cardiomyocytes the standard error of the mean would be 1.1%, which is less than half of the standard deviation of 3.0% found between hearts. As another example, cardiomyocyte peak fluorescence difference within one heart was 0.39 counts per second (cps): for six cardiomyocytes the standard error of the mean was 0.16 cps, which is less than half of the standard deviation of 0.35 cps found between hearts.

Measurement of [Ca2+]i dynamics. Cardiomyocytes were loaded with 3 μM fura-2 AM for 15 min at room temperature (Molecular Probes, Eugene, OR). Fluorescence of fura-2 was monitored at 510 nm and excited with a system (IonOptix, Milton, MA) fitted with optical filters of 405 and 365 nm. This choice of excitation wavelengths provided a linear relationship between fluorescence ratio (R) and free Ca2+ concentration (30). Fluorescence intensities were recorded at 200 Hz as photon counting rates. The value for cardiomyocyte fluorescence background was measured after superfusion with Ca2+-free Tyrode + 2 μM digitonin for 4 min, which released cytosolic fura-2, and the subsequent measure of fluorescence with Ca2+-free Tyrode as superfusate. Background and intracellular compartmentalization of fura-2 for each cardiomyocyte were thereby incorporated into the calculation of R (30).

The recorded cardiomyocyte R transients were analyzed to determine the following characteristics: diastolic R (Rdiast), peak R (Rpeak), Rpeak − Rdiast (Rmax), maximum R increase over time (+dR/dtmax), maximum R decrease over time (−dR/dtmax), time to Rpeak, and time to 50% decline to Rdiast (time to 50% Rdiast). It should be noted that, because there is a linear relationship between R and [Ca2+], the relative values and temporal characteristics for R directly reflect the relative values for the [Ca2+]i transient.

Measurement of sarcomere shortening dynamics. Cardiomyocytes not loaded with fura-2 and of lengths between 90 and 130 μm were examined for sarcomere shortening dynamics. Mean sarcomere length of a cardiomyocyte was calculated by using a fast Fourier Transform of a video image recorded at 240 Hz (IonOptix, Milton, MA). The recorded cardiomyocyte sarcomere shortening transients were analyzed to determine the following characteristics: peak shortening as a percentage of diastolic length, time to peak shortening, maximal shortening velocity normalized to diastolic length (+dL/dtmax + Ldiast), maximal relengthening velocity normalized to diastolic length (−dL/dtmax + Ldiast), and time to 50% relengthening to diastolic length (time to 50% Ldiast).

Analysis

All analyses were performed by using SPSS version 11 (SPSS, Chicago, IL). Each of the eight populations examined was identified for activity as HA or NA, for blood pressure as HT or NT trait, and for sex as M or F. As mentioned earlier, Table 1 characterizes each population by activity, blood pressure, and sex. To differentiate the distinguishing characteristics associated with activity, blood pressure, and sex among the eight populations, we applied a 2(NA, HA)×2(NT, HT)×2(M, F) ANOVA to all variables describing activity, blood pressure, heart rate, rat morphology, and LV protein content. To test for the effects of activity and blood pressure on cardiomyocyte [Ca2+]i dynamics and their frequency responses, descriptive variables were segregated by sex and a 2(NA, HA)×2(NT, HT)×3(2 Hz, 3 Hz, 4 Hz)×6(cardiomyocytes per heart) repeated-measures ANOVA was performed. Similarly, descriptive variables of sarcomere shortening dynamics were segregated by sex, and a 2(NA, HA)×2(NT, HT)×3(2 Hz, 3 Hz, 4 Hz)×4(cardiomyocytes per heart) repeated-measures ANOVA was performed. For purposes of post hoc analyses, all variables for each experimental condition were averaged across the number of cardiomyocytes for each heart to provide a single value per heart, and Student-Newman-Keuls post hoc test was applied to determine significant differences among the WKY, WKHT, WKHA, and SHR populations within the same sex. Statistical significance of ANOVA and post hoc tests are reported at the P < 0.001, P < 0.001, P < 0.01, and P < 0.05 levels. Statistical significance at the P < 0.10 is also presented for variables of which the physiological relevance supports other variables showing statistical significance, thereby minimizing the possibility of Type II (false-negative) errors (35).

RESULTS

Characteristics of Rat Populations

As shown in Table 1, spontaneous locomotor activity in the WKHA and SHR (HA strains) was ~100% greater than that in...
the WKY and WKHT (NA strains) \( (P < 0.0001 \text{ for activity main effect}) \), providing a clear demarcation of the HA trait among the population groups. There were no statistical effects of sex on spontaneous locomotor activity.

Systolic and mean arterial pressures were significantly higher in the WKHT and SHR populations (HT group) compared with WKY and WKHA populations (NT strains) \( (P < 0.0001 \text{ for blood pressure main effect}) \). As one example of this finding, the systolic pressures of M-HT rats, i.e., both M-SHR and M-WKHT, were \( \sim 45 \text{ mmHg} \) higher than that of the respective M-NT rats, i.e., both M-WKHA and M-WKY. Males had significantly higher blood pressures than females within each population \( (P < 0.0001 \text{ for sex main effect}) \), and the degree by which males were more hypertensive than females was greater in the HT group \( (P < 0.0001 \text{ for blood pressure \times sex interaction}) \). This last statistical interaction can be appreciated as the modest 5- to 10-mmHg higher pressures in M-NT relative to F-NT, compared with that in the HT group, where heart rate was only \( \sim 30 \text{ beats/min} \) lower in M-HT relative to F-HT (Table 1).

Body mass was statistically lower in the HA group \( (P < 0.0001) \) and higher in the HT group \( (P < 0.01) \). There was no activity by blood pressure interaction. As expected, males of each population were significantly larger than females by \( \sim 100 – 120 \text{ g} \) \( (P < 0.0001) \).

**Characteristics of Left Ventricles**

Figure 1 shows the characteristics of hearts and lungs obtained from the population of rats used specifically in the present study. Body mass mimicked that reported above for the general population and was lower in the HA group \( (P < 0.0001) \) and lower in the HT group \( (P < 0.0001) \). There was no activity by blood pressure interaction. These results suggest that the hyperactive trait is linked to a reduced vagal tone and/or enhanced sympathetic drive of chronotropy (33), whereas the hypertensive trait is conversely linked to enhanced vagal tone and/or reduced sympathetic drive. There was also a significantly lower heart rate among males of all the populations \( (P < 0.0001 \text{ for sex main effect}) \) with a significant interaction with blood pressure state \( (P < 0.0001 \text{ for blood pressure \times sex interaction}) \). This last interaction can be seen as a greater sex dependence of heart rate observed in the NT group, where heart rate was 50–70 beats/min lower in M-NT relative to F-NT, compared with that in the HT group, where heart rate was only \( \sim 30 \text{ beats/min} \) lower in M-HT relative to F-HT (Table 1).

Fig. 1. Characteristics of rats used in present study. A: lung-to-body mass ratio was not significantly affected by hyperactivity (HA), hypertension (HT), or the combination of HA and HT in either sex. B: left ventricular (LV)-to-body mass ratio was significantly enhanced in the WKHT, WKHA and SHR strains compared with WKY controls. Note that the LV hypertrophic response associated with the HA strains, WKHA, and SHR was greater in the males compared with females. C: abundance of sarcoplasmic reticulum \( \text{Ca}^{2+} \) (SERCA2a) was significantly higher in females compared with males and in HA strains compared with nonhyperactive. D: abundance of phospholamben (PHB) was higher in males compared with females. a, Different from WKY; b, different from WKHT; c, different from WKHA; d, different from SHR of same sex at \( P < 0.05 \) level.
Statistical results for LV-to-body mass ratio included a significant sex main effect \((P < 0.01)\), significant activity main effect \((P < 0.01)\), and significant sex by activity interaction \((P < 0.01)\). Collectively, these results suggest that, although LV-to-body mass ratio was enhanced in males and in HA rats, LV hypertrophy associated with HA was clearly greater in the males than in the females. Figure 1B illustrates this phenomenon as LV hypertrophy in the M-HA compared with M-WKY rats and as a relatively modest LV hypertrophy in the F-HA compared with F-WKY. Statistical results for LV-to-body mass ratio also included a significant blood pressure effect \((P < 0.01)\), which, as expected, reflected an increase in LV mass in the HT group. However, there was also a significant activity by blood pressure interaction \((P < 0.01)\), which indicated an inhibition of the hypertrophic response to hypertension in the HA rats irrespective of sex. Specifically, as shown in Fig. 1B, hypertension in NA rats (WKHT vs. WKY) led to a dramatic increase in the LV-to-body mass ratio, whereas hypertension in HA rats (SHR vs. WKHA) led to only a modest increase in the LV-to-body mass ratio.

As can be seen in Fig. 1C, females possessed about 100% more LV SERCA2a protein compared with males \((P < 0.01)\), and the HA group possessed a marginally greater amount of SERCA2a protein than the NA group \((P < 0.05)\). The greater amount of SERCA2a in females and in HA strains may reflect a heavier reliance on SERCA2a for relaxation and on SR calcium stores for myofilament activation. The ratio PHB to SERCA2a (Fig. 1D) was greater in the males compared with females \((P < 0.01)\), which may reflect a greater degree of SERCA2a inhibition and/or heavier reliance on protein kinase-A activation for modulation of SERCA2a function in males compared with females.

Significant sex differences in morphology, such as body mass and lung-to-body mass ratio, were also found in measures specific to cardiac tissue, such as LV-to-body mass ratio and LV protein content. These strongly sex-dependent findings prompted us to segregate cardiomyocyte data according to sex, as presented below.

**Males: Cardiomyocyte \([\text{Ca}^{2+}]_i\) Dynamics**

Figure 2, A and B, depicts representative fura-2 fluorescence ratio \((R)\) transients recorded in LV cardiomyocytes isolated from M-WKY, M-WKHT, M-WKHA, and M-SHR rats and electrically stimulated at 4 Hz. Parameters of \([\text{Ca}^{2+}]_i\) dynamics at 4 Hz are illustrated in Fig. 3, A–D, and ANOVA results for these parameters are given in Table 2.

Systolic \([\text{Ca}^{2+}]_i\) dynamics were significantly differentiated among the groups by the HA trait as indicated by the significant activity main effects for the parameters \(+dR/dt_{\text{max}}, R_{\text{diff}}\) and time to \(R_{\text{peak}}\) (Table 2). Systolic \([\text{Ca}^{2+}]_i\) function, depicted by higher values for \(+dR/dt_{\text{max}}\) and \(R_{\text{diff}}\) and lower values for time to \(R_{\text{peak}}\) in Fig. 3, A–C, was reduced in the HA strains (M-WKHA and M-SHR) compared with NA (M-WKY and M-WKHT). The lack of an activity by blood pressure interaction is important to note (Table 2) because it dispels the interpretation that differences in systolic \([\text{Ca}^{2+}]_i\) dynamics associated with the HA trait could have been due to any one
population such as the M-WKHT, having singularly influenced the significant activity main effects.

Diastolic \([\text{Ca}^{2+}]_i\) dynamics were also differentiated by the HA trait as indicated by a significant activity main effect for the parameters \(-dR/dt_{max}\) and time to 50% Rdias. Figure 3D illustrates that the time to Rpeak and time to 50% Rdias were also reduced in M-HA strains compared with M-NA, and the effects of HT interacted significantly with HA. HT in the M-WKHT led to enhanced diastolic \([\text{Ca}^{2+}]_i\) function, whereas HT in the M-SHR led to reduced diastolic \([\text{Ca}^{2+}]_i\) function. E and F: systolic sarcomere dynamics, characterized by maximal shortening velocity normalized to diastolic length \((+dL/dt_{max} ÷ L_{dias})\) and peak shortening, were significantly diminished by HT and more so in the M-SHR than in the M-WKHT. G and H: diastolic sarcomere dynamics were significantly reduced in the M-SHR.

Table 2. Comparative cardiomyocyte function in male rats

<table>
<thead>
<tr>
<th>Function</th>
<th>Act BP</th>
<th>Act × BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{Ca}^{2+}]_i) dynamics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(+dR/dt_{max})</td>
<td>0.066</td>
<td>NS</td>
</tr>
<tr>
<td>(R_{diff})</td>
<td>0.019</td>
<td>NS</td>
</tr>
<tr>
<td>Time to Rpeak</td>
<td>0.026</td>
<td>NS</td>
</tr>
<tr>
<td>(-dR/dt_{max})</td>
<td>0.009</td>
<td>NS</td>
</tr>
<tr>
<td>Time to 50% Rdias</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Sarcomere dynamics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(+dL/dt_{max} ÷ L_{dias})</td>
<td>NS</td>
<td>0.015</td>
</tr>
<tr>
<td>Peak shortening</td>
<td>NS</td>
<td>0.035</td>
</tr>
<tr>
<td>Time to peak</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>(-dL/dt_{max} ÷ L_{dias})</td>
<td>NS</td>
<td>0.010</td>
</tr>
<tr>
<td>Time to 50% Ldias</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Fig. 3. Characteristics of male rat cardiomyocyte \([\text{Ca}^{2+}]_i\) and sarcomere dynamics at 4.0 Hz. Results at 2 and 3 Hz were qualitatively similar to these presented at 4 Hz. A and B: systolic \([\text{Ca}^{2+}]_i\), variables maximum R increase over time (+dR/dt_{max}), peak (Rpeak) minus diastolic R (Rdias) (Rdiff), and time to Rpeak were reduced in the M-HA strains compared with M-NA and were significantly enhanced in the M-WKHT. C and D: those \([\text{Ca}^{2+}]_i\), variables reflecting diastolic \([\text{Ca}^{2+}]_i\) function, i.e., time to Rpeak and time to 50% Rdias, were also reduced in M-HA strains compared with M-NA, and the effects of HT interacted significantly with HA. HT in the M-WKHT led to enhanced diastolic \([\text{Ca}^{2+}]_i\) function, whereas HT in the M-SHR led to reduced diastolic \([\text{Ca}^{2+}]_i\) function. E and F: systolic sarcomere dynamics, characterized by maximal shortening velocity normalized to diastolic length (+dL/dt_{max} ÷ L_{dias}) and peak shortening, were significantly diminished by HT and more so in the M-SHR than in the M-WKHT. G and H: diastolic sarcomere dynamics were significantly reduced in the M-SHR. a, Different from WKY; b, different from WKHT; c, different from WKHA; d, different from SHR at \(P < 0.05\) level.

Different from WKY; b, different from WKHT; c, different from WKHA; d, different from SHR at \(P < 0.05\) level.
Males: Cardiomyocyte Sarcomere Dynamics

Figure 2, C and D, depicts representative sarcomere length transients recorded in male rat LV cardiomyocytes not loaded with fura-2. Parameters of sarcomere dynamics are illustrated in Fig. 3, E–H, and ANOVA results are given in Table 2.

Systolic sarcomere dynamics, reflected in the parameters $-dL/dt_{\text{max}} \div L_{\text{dias}}$ and peak shortening, were significantly differentiated among the groups by the HT trait as indicated by significant blood pressure main effects for these parameters (Table 2). Systolic sarcomere dynamics, which would be reflected proportionally to values for $+dL/dt_{\text{max}} \div L_{\text{dias}}$ and peak shortening in Fig. 3, E and F, was reduced in the HT strains (M-WKHT and M-SHR) compared with NT (M-WKY and M-WKHA). It is interesting to note that results for the systolic $[\text{Ca}^{2+}]_{i}$ dynamics would have predicted enhanced systolic sarcomere dynamics in the M-WKHT strain compared with controls. The lack thereof in combination with the significant reduction in systolic sarcomere dynamics in the M-SHR (Fig. 3, E and F) suggests that the myofilament responsiveness to systolic $[\text{Ca}^{2+}]_{i}$ dynamics were significantly diminished in the HT groups.

Results for one parameter of diastolic sarcomere dynamics, namely $-dL/dt_{\text{max}} \div L_{\text{dias}}$, was differentiated by HT trait as indicated by a significant blood pressure main effect, which must be interpreted in the light of a trend for a significant activity by blood pressure interaction (Table 2). As illustrated in Fig. 2D, the maximum slope of sarcomere relengthening, i.e., $-dL/dt_{\text{max}} \div L_{\text{dias}}$, was significantly reduced in the M-SHR compared with the other groups and likely accounts for the blood pressure main effect and the activity by blood pressure interaction found for this parameter. The parameters of diastolic sarcomere dynamics, $-dL/dt_{\text{max}} \div L_{\text{dias}}$ and time to 50% $L_{\text{dias}}$, demonstrated similar and consistent trends for activity by blood pressure interactions (Table 2). This interaction is illustrated in Fig. 3H by the higher values for time to 50% $L_{\text{dias}}$ (corresponding to poor diastolic sarcomere dynamics) in the M-SHR, which is both HT and HA, and lower values for time to 50% $L_{\text{dias}}$ (corresponding to higher diastolic sarcomere dynamics) in the M-WKHT, which is HT and NA.

Females: Cardiomyocyte $[\text{Ca}^{2+}]_{i}$ Dynamics

Figure 4, A and B, depicts representative fura-2 R transients recorded in female rat LV cardiomyocytes. Parameters of $[\text{Ca}^{2+}]_{i}$ dynamics are illustrated in Fig. 5, A–D, and ANOVA results for these parameters are given in Table 3.

Systolic $[\text{Ca}^{2+}]_{i}$ dynamics, $+dR/dt_{\text{max}}$, and $R_{\text{diff}}$ were differentiated by the HA and HT traits indicated by statistically significant activity and blood pressure main effects (Table 3). As illustrated in Fig. 5, A and B, values for $+dR/dt_{\text{max}}$ and $R_{\text{diff}}$ were diminished in the HA strains (F-WKHA and F-SHR) compared with NA (F-WKY and WKHT) and enhanced in the HT strains (F-WKHT and F-SHR) compared with NT (F-WKY and F-WKHA). The temporal parameter time to $R_{\text{peak}}$ was not found to be dependent on activity and/or blood pressure.
Diastolic [Ca\textsuperscript{2+}] dynamics, reflected in time to 50\% \textit{R}\textsubscript{dias}, were significantly differentiated by the HA trait (Table 3). As illustrated in Fig. 5, time to 50\% \textit{R}\textsubscript{dias} was reduced in the HA strains compared with NA.

**Females: Cardiomyocyte Sarcomere Dynamics**

Figure 4, C and D, depicts representative sarcomere length transients recorded in female rat LV cardiomyocytes not loaded with fura-2. Parameters of sarcomere dynamics are illustrated in Fig. 5, E–H, and ANOVA results are given in Table 3. Systolic sarcomere dynamics, reflected in \textit{dL}/\textit{dt}_{\text{max}}, \textit{R}_{\text{diff}}, and time to \textit{R}_{\text{peak}} were not differentiated by the HA and/or HT traits in these female rat cardiomyocytes.

The temporal characteristics of diastolic sarcomere dynamics, time to 50\% \textit{L}\textsubscript{dias}, and to some degree time to peak were significantly differentiated by HA as indicated by significant activity main effects (Table 3). As illustrated in Fig. 5, H, time to 50\% \textit{L}\textsubscript{dias} was prolonged in the HA strains compared with NA. This result may be due to diastolic [Ca\textsuperscript{2+}] dynamics, which were also reduced by HA (Table 3 and Fig. 5, D).

**Frequency Responses**

For the sake of brevity we have so far presented representative data at only 4 Hz, because differences in parameter values among the populations were qualitatively similar at the three frequencies (2, 3, and 4 Hz) examined in this study. Nevertheless, some parameter values changed significantly with stimulation frequency. As illustrated in Fig. 6A for male and Fig. 6B for female rat cardiomyocytes, the diastolic [Ca\textsuperscript{2+}] parameter time to 50\% \textit{R}\textsubscript{dias} shortened significantly (\textit{P} < 0.001 frequency main effect in both sexes) as stimulation frequency increased, i.e., a positive relaxation-frequency response. In addition, repeated-measures ANOVA for time to

---

**Table 3. Comparative cardiomyocyte function in female rats**

<table>
<thead>
<tr>
<th>[Ca\textsuperscript{2+}] Dynamics</th>
<th>Act</th>
<th>BP</th>
<th>Act × BP</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{dR}/\textit{dt}_{\text{max}}</td>
<td>(0.082)</td>
<td>0.045</td>
<td>NS</td>
<td>Sys</td>
</tr>
<tr>
<td>\textit{R}_{\text{diff}}</td>
<td>&lt;0.001</td>
<td>0.011</td>
<td>NS</td>
<td>Sys</td>
</tr>
<tr>
<td>Time to \textit{R}_{\text{peak}}</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>Sys, Dia</td>
</tr>
<tr>
<td>\textit{dL}/\textit{dt}_{\text{max}}</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>Dia</td>
</tr>
<tr>
<td>Time to 50% \textit{R}_{\text{dias}}</td>
<td>0.007</td>
<td>NS</td>
<td>NS</td>
<td>Dia</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sarcomere dynamics</th>
<th>Act</th>
<th>BP</th>
<th>Act × BP</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{dL}/\textit{dt}_{\text{max}}</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>Sys</td>
</tr>
<tr>
<td>Peak shortening</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>Sys</td>
</tr>
<tr>
<td>Time to peak</td>
<td>0.045</td>
<td>NS</td>
<td>NS</td>
<td>Dia</td>
</tr>
<tr>
<td>\textit{dL}/\textit{dt}_{\text{max}}</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>Dia</td>
</tr>
<tr>
<td>Time to 50% \textit{L}_{\text{dias}}</td>
<td>0.049</td>
<td>NS</td>
<td>NS</td>
<td>Dia</td>
</tr>
</tbody>
</table>

\textit{P} values for significant results of repeated-measures ANOVA are reported of significant main effects of Act, BP, and their interactions. Values of \textit{P} < 0.1 are shown only when in support of other variables describing a similar function: Sys, systolic; Dia, diastolic.

---

**Fig. 5. Characteristics of female rat cardiomyocyte [Ca\textsuperscript{2+}], and sarcomere dynamics at 4.0 Hz. Results at 2 and 3 Hz were qualitatively similar to those presented at 4 Hz. A and B: systolic [Ca\textsuperscript{2+}], variables +dR/dt_{\text{max}}, R_{\text{diff}}, and time to R_{\text{peak}} were reduced by HA and were significantly enhanced in the F-WKHT. C and D: those variables reflecting diastolic [Ca\textsuperscript{2+}], function, i.e., time to R_{\text{peak}} and time to 50\% \textit{R}_{\text{dias}} were significantly reduced by HA. E and F: systolic sarcomere dynamics, i.e., +dL/dt_{\text{max}} + L_{\text{dias}} and peak shortening, were similar among all four groups of females. G and H: temporal characteristics of diastolic sarcomere dynamics were enhanced in the F-WKHT and reduced in the F-SHR. a, Different from WKY; b, different from WKHT; c, different from WKHA; d, different from SHR at \textit{P} < 0.05 level.**
50% $R_{\text{dias}}$ demonstrated a frequency by activity interaction that was nearly significant ($P = 0.059$) for males and highly significant ($P < 0.001$) for females. Collectively, these results for time to 50% $R_{\text{dias}}$ suggest that the frequency response of Ca$^{2+}$ uptake by SERCA2a is enhanced in the HA groups. It is important to note that the relatively enhanced frequency response of Ca$^{2+}$ uptake by SERCA2a in the HA groups was accompanied by and may be due to the relatively higher abundance of SERCA2a protein in the HA groups (Fig. 1C).

Because the diastolic [Ca$^{2+}$]$_i$ dynamics, like that reflected in time to 50% $R_{\text{dias}}$, strongly influence diastolic sarcomere dynamics, a comparison of the frequency responses for time to 50% $R_{\text{dias}}$ and the frequency responses for time to 50% $L_{\text{dias}}$ is presented to illustrate a possible contribution to sarcomere relengthening that is calcium independent. Time to 50% $L_{\text{dias}}$ (Fig. 6C for males and 6D for females) was significantly reduced by stimulation frequency in both sexes. However, the frequency response of time to 50% $L_{\text{dias}}$ was significantly blunted in the WKHA and WKHT groups in the male rat cardiomyocytes, indicated by the frequency by activity by blood pressure interaction and the post hoc analyses. The frequency response of time to 50% $L_{\text{dias}}$ were not reflected in the frequency response of time to 50% $R_{\text{dias}}$ in the males (Fig. 6A). In the female rat cardiomyocytes the frequency response for time to 50% $L_{\text{dias}}$ was not differentiated by HA and/or HT, whereas that for time to 50% $R_{\text{dias}}$ was significantly influenced by HA (Fig. 6B). These data suggest that the frequency response of diastolic sarcomere dynamics in both sexes was significantly influenced by something other than diastolic [Ca$^{2+}$]$_i$ dynamics. We anticipate that protein isoform profiles and protein phosphorylation states in the myofilaments play significant roles in calcium-independent sarcomere relengthening in these rat cardiomyocytes and are differentiated among the HA and HT traits.

**DISCUSSION**

Characteristics of cardiomyocyte function were differentially affected in three naturalistic models of LV hypertrophy associated with HA alone, HT alone, and the combination of HA and HT, namely the WKHA, WKHT, and SHR strains, respectively. Surprisingly, we found significantly enhanced systolic and diastolic [Ca$^{2+}$]$_i$ dynamics and relatively normal systolic and enhanced diastolic sarcomere dynamics in the WKHT, which is HT and not HA, compared with WKY controls (Figs. 3 and 5). High peripheral vascular resistance underlies HT in the WKHT, as it does in the SHR (23, 31), and likely, although not shown definitively, contributed to the LV hypertrophic response observed in both M and F WKHT (Fig. 1B). The traits of hyperactivity and hyperreactivity to stress, however, are absent in the WKHT and therefore did not contribute to LV hypertrophy or to enhanced LV cardiomyocyte function in the WKHT strain. From the data presented in the current study, we speculate that a reduction in the myofilament sensitivity to [Ca$^{2+}$]$_i$ may be an underlying modification of cardiac myofilaments in the WKHT. Such an effect on myofilament characteristics in the WKHT would improve diastolic function in the face of HT and would therefore be worthy of future study. We further propose that the WKHT at the ages studied here may serve as a model of compensated LV hypertrophy and function associated with hypertension without the confounding factors of hyperactivity and hypertreactivity to stress found in the SHR.

In contrast to the rather straightforward assessment of LV cardiomyocyte function associated with HT alone in the WKHT, LV cardiomyocyte function associated with HA alone and in combination with HT is less straightforward to evaluate and in some cases highly dependent on sex. Among the most important observations associated with the HA strains are the following. First, characteristics of systolic and diastolic [Ca$^{2+}$]$_i$ dynamics demonstrated reduced calcium regulatory function in the HA strains (WKHA and SHR) compared with NA strains.
and SHR of the same sex (Figs. 3, 4, 5, and 6) demonstrate important sex differences in calcium regulatory mechanisms that are independent of sex. For example, in female SHR, SERCA2a abundance is higher compared to male SHR, which is consistent with LV hypertrophy in the absence of HT. Considering that human males but not females with hypertensive personalities are at higher risk of developing LV hypertrophy due to HT (27), the four strains examined in the current study may serve well in studies of LV hypertrophy following HT and modified by HA and male sex.

ACKNOWLEDGMENTS

This work was supported in part by National Heart, Lung, and Blood Institute Grant RO1 HL-52087 (to B. Palmer, Z. Chen, R. Lachapelle, and M. LeWinter).

REFERENCES


