Mild renal hypertension alters run training effects on the frequency response of rat cardiomyocyte mechanics

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Palmer, Bradley M., Eric A. Mokelke, Anne M. Thayer, and Russell L. Moore. Mild renal hypertension alters run training effects on the frequency response of rat cardiomyocyte mechanics. J Appl Physiol 95: 1799–1807, 2003.—We examined the effects of run training on the frequency dependence of cardiomyocyte mechanics and intracellular calcium concentration ([Ca\(^{2+}\)]_{i}) dynamics in rats with mild renal hypertension. Male Fischer 344 rats aged 2–3 mo underwent a sham operation or stenosis of the left renal artery, which increased systolic blood pressure 20–30 mmHg. Half of the rats in each group underwent treadmill run training for >16 wk. Isolated cardiomyocytes were paced at 1.0 and 0.2 Hz in 2 mM external Ca\(^{2+}\) concentration at 29°C. Under these conditions, negative frequency responses, i.e., decreased value with increased frequency, were recorded for peak shortening, shortening velocity, and the integral of the [Ca\(^{2+}\)]_{i} transient in both groups. Run training amplified the negative frequency response for the integral of the [Ca\(^{2+}\)]_{i} transient in both groups, but it amplified the negative frequency response for the shortening dynamics only in the normotensive sham-operated and not in the hypertensive rats. These results, as well as others for relaxation parameters, suggest that renal hypertension altered the effects of run training on the frequency response for cardiomyocyte contractile apparatus and/or passive mechanical properties, which respond to [Ca\(^{2+}\)]_{i}.

The force-frequency relationship; relaxation-frequency relationship; calcium; treadmill

The force-frequency relationship of cardiac muscle refers to the change in the force of contraction induced by a change in the frequency of contraction. The force-frequency relationship in instrumented and conscious subjects has consistently been observed to be positive, i.e., increased force with increased frequency, for all species yet examined due in part to its mediation in vivo by β-adrenergic stimulation (25) and by nitric oxide (15). Under laboratory conditions, however, myocardium isolated from mice and rats often (4–6, 18), although not always (2), displays a negative force-frequency relationship, whereas that from higher animals and humans normally displays a positive relationship (4–6, 25). The integrity of intracellular protein kinase signaling pathways, which include a cAMP response element modulator (14), plays a significant role in determining the capacity of the myocardium to respond to changes in frequency with modifications to contraction and relaxation (9, 25). However, other mechanisms underlying the frequency responses for contraction and relaxation also include the passive mechanical properties of the myocardium, the response of the contractile apparatus to activation by Ca\(^{2+}\), and intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]_{i}) dynamics (12, 29).

The combined influence of Ca\(^{2+}\) regulatory proteins and protein kinase signaling pathways on [Ca\(^{2+}\)]_{i} dynamics has so far provided the primary explanation for physiological and pathological force-frequency relationships (6, 25). A positive force-frequency relationship is thought to occur when experimental conditions and Ca\(^{2+}\) regulatory mechanisms favor increased Ca\(^{2+}\) influx via L-type Ca\(^{2+}\) channels and increased Ca\(^{2+}\) uptake into and release from the sarcoplasmic reticulum (SR) as the frequency of contraction rises and Ca\(^{2+}\)-dependent protein kinase signaling pathways activate (6, 25, 30). Evidence is mounting to suggest that a negative force-frequency relationship occurs when, as the frequency of contraction rises, experimental conditions and Ca\(^{2+}\) regulatory mechanisms lead to a comparatively insufficient activation of protein kinase signaling pathways (1), a diminished capacity for modification of SR Ca\(^{2+}\) uptake rate (4–6, 30), an increased efflux of Ca\(^{2+}\) from cardiomyocytes (4), and/or increased proportions of refractory L-type and SR Ca\(^{2+}\) channels (2, 18). In failing human left ventricle (LV), for example, the reduced density, sensitivity, and basal activity of β-adrenergic receptors and receptor-associated proteins are at least partially responsible for the abnormal force-frequency relationship observed in vivo and in vitro. Refer to Alpert et al. (1) and Hasenfuss and Pieper (13) for excellent reviews of the contributions of the β-adrenergic signaling pathway, SR Ca\(^{2+}\)-ATPase (SERCA2), phospholamban, Na\(^+\)/Ca\(^{2+}\) exchanger (NCX1) and other Ca\(^{2+}\)-handling proteins on the altered force-frequency relationship in failing human LV.

A reduction in SR Ca\(^{2+}\) load with an increasing frequency of contraction has been shown to accompany the negative frequency response of rat myocardial force (4–6, 30) and cardiomyocyte shortening (4, 30) and...
likely underlies the negative frequency response of isolated rat LV pressure development (16, 19). Experimental pressure overload and renal hypertension in the rat further diminishes SR Ca\(^{2+}\) load and diastolic function largely because of reduced activity of SERCA2 (7), whose modulation by phosphorylation of PHB is then limited by the accompanying depression of β-adrenergic responsiveness (22). Collectively, these characteristics of negative force-frequency relationship, diminished diastolic function, and depressed β-adrenergic responsiveness provide a rationale for the use of rat myocardium after pressure overload or renal hypertension as a model for the study of similar characteristics observed in failing human myocardium.

In contrast to the effects of experimental hypertension on the force-frequency relationship and β-adrenergic responsiveness, exercise training in rats has been shown to make positive the frequency response of cardiomyocyte shortening (33), improve diastolic function (34), and in some accounts increase the β-adrenergic responsiveness in isolated myocardium (27, 31). It has been proposed for some time that the partial restoration of cardiac functions, which have been compromised during experimental hypertension, may be possible through physical exercise (12, 26, 27). Indeed, run training has been shown to maintain otherwise diminishing cardiac function in renal hypertensive rats (26), increase the density and/or activity of SR Ca\(^{2+}\) regulatory proteins that are compromised in chronic hypertension and heart failure (3, 7) and rescue α-adrenergic responsiveness in rats with mild renal hypertension (16). In addition, run training has been shown to increase the fraction of SR Ca\(^{2+}\) released during a single contraction in cardiomyocytes from rats with mild renal hypertension (24) and may therefore be expected to encourage a positive force-frequency response.

In this study it was hypothesized that run training in male Fischer 344 rats would encourage positive frequency responses for cardiomyocyte shortening and [Ca\(^{2+}\)]\(_i\) dynamics. We report that run training instead amplified a negative frequency response for cardiomyocyte shortening dynamics in normotensive rats but not in the renal hypertensive rats. On examining the frequency response for underlying [Ca\(^{2+}\)]\(_i\) dynamics, run training amplified the negative frequency response for the integral of the [Ca\(^{2+}\)]\(_i\) transient in both the normotensive and renal hypertensive rats. These results, as well as others pertaining to diastolic function, provide evidence that renal hypertension significantly altered the normal effects of run training on the frequency responses for the cardiomyocyte contractile apparatus and/or passive mechanical properties, which dictate shortening and lengthening dynamics in response to the [Ca\(^{2+}\)]\(_i\) transient.

**METHODS**

**Animal preparation.** Male Fischer 344 rats 2–3 mo old were housed in a 12:12-h light-dark cycle, given standard rat chow and water ad libitum, and randomly assigned to a normotensive (NT, n = 14) or hypertensive (HT, n = 14) group. Rats in the HT group underwent stenosis of the left renal artery using surgical hemoclips of internal diameter 0.17–0.23 cm to produce a Goldblatt (2 kidney-1 clip) model of renal hypertension as described previously (16, 24). Rats of the NT group underwent a sham operation.

After a 4-wk recovery from surgery, systolic blood pressure was recorded in each rat by using a tail cuff sphygmomanometer (Gould Instruments, Cleveland, OH) to verify the blood pressure states of the NT and HT groups. Half of the rats in each group were randomly assigned to a training (Tr) or sedentary (Sed) subgroup. Rats of the Tr subgroups were treadmill trained for at least 16 wk, 5 days/wk. Running duration and intensity began at 10 min/day at 20 m/min, respectively, and were progressively increased over 8 wk to 20 min at 20 m/min plus 40 min at 26 m/min. This final duration and intensity regimen was maintained for 8–14 wk. 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 Colorado, Boulder Campus.

**LV cardiomyocyte isolation.** LV cardiomyocytes were obtained from the LV septal and free wall by use of methods described previously (21). All chemicals and reagents were obtained from Sigma Chemical (St. Louis, MO) except where noted. In brief, animals were heparinized (250 U ip) and then anesthetized with pentobarbital sodium (35 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 was retrogradely perfused by using a modified Langendorff perfusion apparatus, which delivered a bicarbonate-based modified Krebs-Henseleit buffer, a nominally Ca\(^{2+}\)-free solution, and solution containing collagenase (Worthington, Freehold, NJ) and hyaluronidase. All solutions were maintained at pH 7.4 and 37°C and were bubbled with 95% O\(_2\)-5% CO\(_2\) gas. The atria and right ventricular free wall were removed, leaving the LV free wall and septum, which were minced and placed in a collagenase and hyaluronidase solution. Isolated cardiomyocytes were suspended in bicarbonate-based medium 199, seeded onto laminin-coated 2-cm-diameter glass coverslips, and placed in an incubator at 37°C and 5% CO\(_2\)-balance room air.

**Experimental protocol.** After at least 2 h incubation, coverslips were removed from the media and used to form the bottom plate of a custom flow-through chamber. The chamber was placed on the stage of an inverted microscope (Nikon Instruments, Provo, UT) and recorded at 200 Hz by using an analog-to-digital converter. The effective width was calculated as area/length.

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). Shortening and [Ca\(^{2+}\)] dynamics were examined after steady-state contractile function was achieved at 1.0 Hz followed by 0.2 Hz.

**Measurement of shortening dynamics.** Cardiomyocytes not loaded with fura-2 were examined for shortening dynamics. The positions of cardiomyocyte edges were determined by using a video edge-detection device (Crescent Electronics, Provo, UT) and recorded at 200 Hz by using an analog-to-digital converter. The recorded cardiomyocyte shortening transients were analyzed by custom-made software to determine the following characteristics: peak shortening as a percentage of resting length, time to peak shortening, maxi-
normal shortening velocity normalized to resting length, and maximal relengthening velocity normalized to resting length.

Measurement of \([Ca^{2+}]_i\) dynamics. Cardiomyocytes were exposed to 2 \(\mu M\) fura-2 AM (Molecular Probes, Eugene, OR) for 5 min in the incubator at 37°C. Fluorescence of fura-2 was monitored at 510 nm and excited with a system (IonOptix, Milton, MA) fitted with optical filters of 400 and 560 nm. This choice of excitation wavelengths provided a linear relationship between fluorescence ratio (R) and free Ca\(^{2+}\) concentration (23). Fluorescence intensities were recorded at 200 Hz as photon counting rates by use of a personal computer. The value for cardiomyocyte fluorescence background was determined for at least one cardiomyocyte per isolation by superfusion with Ca\(^{2+}\)-free Tyrode + 2 \(\mu M\) digitonin for 4 min, which released cytosolic fura 2, and the subsequent measure of fluorescence with Ca\(^{2+}\)-free Tyrode as superfusate. Average background and fluorescence of fura-2 residing in intracellular areas inaccessible to cytosolic Ca\(^{2+}\) were estimated from the data acquired from the cardiomyocytes exposed to digitonin. Background and intracellular compartmentalization of fura-2 for each cardiomyocyte were then estimated on the basis of the relative dimensions of the cardiomyocyte and were therefore incorporated into the calculation of R (23).

The recorded cardiomyocyte R transients were analyzed to determine the following characteristics: resting R \((R_{rest})\), peak minus resting R \((R_{peak})\), the integral of the R transient \((R_{int})\), time to peak R, and the falling exponential rate constant \((k_{fall})\) determined by nonlinear least-squares fitting of a single-exponential function to the falling portion of the recorded R transient (24). It should be noted that, because of a linear relationship between R and \([Ca^{2+}]_i\), the relative values and temporal characteristics for R directly reflected those for the \([Ca^{2+}]_i\) transient.

Analysis. All analyses were performed using SPSS version 6.1 (SPSS). All data are reported as means ± SE. To test for morphological differences between the Nt and Ht groups and find significant variables, Duncan’s multiple range test was performed. ANOVA results was reported at the \(P < 0.05\) level, although \(P < 0.1\) was interpreted to indicate neither a significant difference nor similarity (32).

RESULTS

Animal model. Characteristics of the animal model used in this study are presented in Table 1. Renal hypertension was characterized by a significantly increased systolic blood pressure of ~20–30 mmHg, increased right kidney mass (~35%), and decreased left kidney mass (~40%). These results in the Ht group collectively suggested that stenosis of the left renal artery successfully reduced blood flow to the left kidney and led to significant physiological modifications consistent with a rat model of renal hypertension described by others (16, 22, 24, 30). Run training induced an increased citrate synthase activity of the plantaris muscle (~18%), decreased body mass (~12%), and increased cardiomyocyte length (~5%). These results in the Tr subgroups suggested that both peripheral and cardiac-specific adaptations to run training were similar to those reported previously (16, 17, 21, 24).

The isolation of cardiomyocytes precluded our measuring ventricular masses for statistical comparison. However, it is interesting to note that we did not find cardiomyocyte width in the Ht group to be increased compared with that in the Nt group. It has been most often reported that renal hypertension causes myocardial hypertrophy characterized by wider cardiomyocytes (22, 30, 35) when systolic blood pressure has been raised >50 mmHg (8, 20). However, other two-kidney, one-clip models of renal hypertension in male Fischer 344 and male Sprague-Dawley rats, which have shown increases in systolic blood pressure of only 20–30 mmHg, do not develop cardiac hypertrophy (16, 20) or increased cardiomyocyte width or area (24). Our rat model of renal hypertension would therefore be best characterized as mild or moderate renal hypertension without cardiac hypertrophy.

Frequency response of cardiomyocyte shortening dynamics. Figure 1 depicts representative shortening transients recorded from Nt+Sed (A), Nt+Tr (B), Ht+Sed (C), and Ht+Tr (D) cardiomyocytes stimulated

Table 1. Animal morphology characteristics, plantaris muscle CSA, and cardiomyocyte dimensions

<table>
<thead>
<tr>
<th></th>
<th>Nt+Sed</th>
<th>Nt+Tr</th>
<th>Ht+Sed</th>
<th>Ht+Tr</th>
<th>ANOVA</th>
</tr>
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<tbody>
<tr>
<td>Number of rats</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Body mass, g</td>
<td>397 ± 13</td>
<td>350 ± 6†</td>
<td>407 ± 9‡</td>
<td>345 ± 9§</td>
<td>*tr</td>
</tr>
<tr>
<td>Tibia length, mm</td>
<td>44.4 ± 0.3</td>
<td>43.4 ± 0.5</td>
<td>45.2 ± 0.4</td>
<td>44.0 ± 0.5</td>
<td>*tr</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>150 ± 4</td>
<td>152 ± 3</td>
<td>177 ± 5†</td>
<td>169 ± 3‡</td>
<td>*bp</td>
</tr>
<tr>
<td>Left kidney/tibia, mg/mm</td>
<td>27.2 ± 10</td>
<td>27.5 ± 0.2</td>
<td>16.5 ± 4.7‡</td>
<td>15.6 ± 3.9‡</td>
<td>*bp</td>
</tr>
<tr>
<td>Right kidney/tibia, mg/mm</td>
<td>26.7 ± 0.9</td>
<td>27.1 ± 0.3</td>
<td>35.8 ± 3.5‡</td>
<td>35.2 ± 2.2‡</td>
<td>*bp</td>
</tr>
<tr>
<td>CSA, mmol-min⁻¹·g protein⁻¹</td>
<td>83.2 ± 4.2</td>
<td>99.6 ± 4.7</td>
<td>77.8 ± 2.3</td>
<td>97.6 ± 3.3§</td>
<td>*tr</td>
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<td>Number of cardiomyocytes</td>
<td>108.7 ± 18</td>
<td>114.9 ± 1.6§</td>
<td>112.9 ± 1.5</td>
<td>116.6 ± 1.6§</td>
<td>*tr</td>
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<tr>
<td>Cardiomyocyte length, µm</td>
<td>145</td>
<td>130</td>
<td>120</td>
<td>180</td>
<td>*tr</td>
</tr>
<tr>
<td>Cardiomyocyte width, µm</td>
<td>31.5 ± 0.7</td>
<td>31.7 ± 0.7</td>
<td>29.8 ± 0.5</td>
<td>30.1 ± 0.5</td>
<td>*bp</td>
</tr>
<tr>
<td>Cardiomyocyte area, µm²</td>
<td>3,413 ± 88</td>
<td>3,625 ± 88</td>
<td>3,358 ± 68</td>
<td>3,580 ± 71§</td>
<td>*tr</td>
</tr>
</tbody>
</table>

Values are means ± SE. Nt, normotensive; Ht, hypertensive; Sed, sedentary; Tr, run trained; CSA, citrate synthase activity. *bp, Significant blood pressure main effect (\(P < 0.05\)); †tr, significant training main effect (\(P < 0.05\)). Post hoc differences (\(P < 0.05\)). ‡ different from Nt+Sed; † different from Nt+Tr; § different from Ht+Sed.
Cardiomyocytes of all four subgroups demonstrated a significant increase in peak shortening when pacing frequency was reduced to 0.2 Hz and therefore displayed the negative frequency response expected for rat cardiomyocytes under these experimental conditions (6, 30). The frequency response of peak shortening was not found to be dependent on the blood pressure state or on the training state of the rats, as indicated by the lack of statistical significance for the blood pressure × frequency and training × frequency interactions (Fig. 2A). There was, however, a statistically significant blood pressure × training × frequency interaction for peak shortening. This interaction is illustrated in Fig. 2A, where the peak shortening of the Nt+Tr subgroup was significantly less than that of Nt+Sed at 1.0 Hz although not at 0.2 Hz, and no similar trend was evident between the Ht+Tr and Ht+Sed. Therefore, run training amplified the
negative frequency response for cardiomyocyte peak shortening in the Nt group but not in the Ht group.

Time to peak shortening at both 1.0 and 0.2 Hz was significantly lowered by run training in the Nt group but not in the Ht group (Fig. 2B). It is noteworthy that run training reduced the time to peak shortening in the Nt group to a value similar to that in the Ht+Sed and Ht+Tr subgroups; therefore, renal hypertension alone induced a reduction in time to peak shortening compared with Nt and thereby may have inhibited any further effect of run training on cardiomyocyte time to peak shortening. With regard to the frequency response, time to peak shortening for all four subgroups tended to rise with the lowering of pacing frequency (Fig. 2B). Cardiomyocytes of the Nt group were significantly more sensitive to the pacing frequency than those from the Ht group, as indicated by a significantly more sensitive blood pressure × frequency interaction. However, there was no differential effect of run training on the frequency response of time to peak shortening in the Nt and Ht groups.

Both maximal shortening velocity and maximal re-lengthening velocity displayed negative frequency responses (Fig. 2, C and D). However, there was no direct dependence of these frequency responses to blood pressure or training states. Run training amplified the negative frequency response of shortening velocity and re-lengthening velocity in the Nt group but not in the Ht group, as indicated by the statistically significant blood pressure × training × frequency interactions for these variables. These three-way interactions can be visualized in Fig. 2, C and D, as the differences in the slopes of the lines representing the frequency responses of the four subgroups. For example, the slope of the Nt+Tr subgroup is steeper than that for the Nt+Sed and indicates an amplified negative frequency response induced by run training in the Nt group. In contrast, the slope of the Ht+Tr subgroup is not as steep as that of the Ht+Sed and indicates a diminished negative frequency response induced by run training.

The negative frequency responses observed with peak shortening, maximal shortening velocity, and maximal re-lengthening velocity collectively suggested that there must be a concomitant negative frequency response for either 1) the contractile apparatus and passive mechanical properties that respond to the [Ca\(^{2+}\)] transient and/or 2) the underlying [Ca\(^{2+}\)] transient. As reported below, we investigated the latter possibility.

**Frequency response of cardiomyocyte [Ca\(^{2+}\)] dynamics.** Figure 3 depicts representative R transients recorded from Nt+Sed, Nt+Tr, Ht+Sed, and Ht+Tr cardiomyocytes stimulated at 1.0 Hz and 0.2 Hz. The reduction in pacing frequency from 1.0 Hz to 0.2 Hz elicited an increase in Rdiff and in Rint for all four cardiomyocyte subgroups, as indicated by the statistically significant frequency main effects for these variables (Fig. 4, A and B). The negative frequency responses for Rdiff and Rint imply a negative frequency response for SR Ca\(^{2+}\) release, which is dependent on the SR Ca\(^{2+}\) load, the proportion and conductance of excitable SR Ca\(^{2+}\) release channels, the sensitivity of the SR release channels to Ca\(^{2+}\)-induced Ca\(^{2+}\) release, and the trigger for Ca\(^{2+}\)-induced Ca\(^{2+}\) release provided by the inward L-type Ca\(^{2+}\) current (6). It should be noted that a negative frequency response for SR Ca\(^{2+}\) load has been previously reported for cardiomyocytes of male Fischer 344 rats studied under the same laboratory conditions (30).
The frequency response for \( R_{\text{diff}} \) was not found to be significantly dependent on blood pressure, run training, or the combination of blood pressure and run training (Fig. 4A). In contrast, there was a statistical tendency for a significant training \( \times \) frequency interaction \((P = 0.066)\) for \( R_{\text{int}} \), which demonstrated at least the possibility that run training amplified the frequency response for \( R_{\text{int}} \) \((32)\). Indeed, the post hoc analysis of \( R_{\text{int}} \) implied that the integral of the [Ca\(^{2+}\)] transient was more sensitive to pacing frequency in the Nt + Tr and Ht + Tr subgroups than in their respective Nt + Sed and Ht + Sed subgroups. Therefore, the negative frequency response for \( R_{\text{int}} \) was amplified by run training.

The integral of the [Ca\(^{2+}\)], transient, as indicated by \( R_{\text{int}} \), would be expected to directly influence peak cardiomyocyte shortening \((29)\). In the Nt cardiomyocytes, for example, the frequency response for \( R_{\text{int}} \) (Fig. 4B) mirrors that for peak cardiomyocyte shortening (Fig. 2A) in both the Nt + Sed and Nt + Tr subgroups. In the Ht cardiomyocytes, however, the frequency response for \( R_{\text{int}} \) mirrors that for peak shortening only in the Ht + Sed subgroup and not in the Ht + Tr subgroup. This observation suggests that the frequency-dependent characteristics of the contractile apparatus and/or of the passive mechanical elements responding to [Ca\(^{2+}\)], in the Ht cardiomyocytes were altered by run training in a manner dissimilar to that in the Nt.

At both 1.0 and 0.2 Hz, time to peak R was significantly prolonged by run training in the Ht group but not in the Nt (Figs. 3 and 4C). Run training during renal hypertension therefore had a significant effect on those Ca\(^{2+}\) regulatory mechanisms collectively responsible for the rate of [Ca\(^{2+}\)] rise; specifically, run training may have reduced SR Ca\(^{2+}\) load, the proportion and conductance of excitable SR Ca\(^{2+}\) release channels, the sensitivity of the SR release channels to Ca\(^{2+}\)-induced Ca\(^{2+}\) release, and/or the inward L-type Ca\(^{2+}\) current in the Ht group. With regard to frequency response, the variable time to peak R was sensitive to pacing frequency, but this sensitivity to pacing frequency was not dependent on blood pressure state, training state, or the combination of blood pressure and training (Fig. 4C).

The rate of [Ca\(^{2+}\)] fall, \( k_{\text{fall}} \), was reduced with the reduction in pacing frequency in all four subgroups (Figs. 4D) and was therefore the only variable that demonstrated a positive frequency response under these laboratory conditions. The representative [Ca\(^{2+}\)] transients in Fig. 3 visually attest to a reduced rate of Ca\(^{2+}\) uptake during pacing at 0.2 Hz compared with that at 1.0 Hz. Because \( k_{\text{fall}} \) indicates the sum of the rates of SERCA2 and NCX1 activity, the positive frequency response for \( k_{\text{fall}} \) was likely due in part to a frequency-dependent enhancement of SERCA2 activity via the phosphorylation of phospholamban by Ca\(^{2+}\)-dependent protein kinase signaling pathways \((6, 25)\). Run training significantly amplified the frequency response of \( k_{\text{fall}} \) in the Ht group but did not appreciably change the frequency response of \( k_{\text{fall}} \) in the Nt group as indicated by the statistically significant blood pressure \( \times \) training \( \times \) frequency interaction (Fig. 4D). As will be discussed in more detail below, the sharp contrast between the positive frequency response for \( k_{\text{fall}} \) (Fig. 4D) and the negative frequency response for relengthening velocity (Fig. 2D) implies that the frequency response of mechanical relengthening is strongly influenced by the contractile apparatus and/or the passive mechanical elements that respond to [Ca\(^{2+}\)] dynamics.

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Frequency responses of contractile apparatus and passive mechanics. The mechanical sensitivity of the cardiomyocyte contractile apparatus and the passive mechanical elements to \([\text{Ca}^{2+}]_i\) dynamics was calculated in two ways: 1) the ratio of cardiomyocyte peak shortening to \(R_{\text{ext}}\) to indicate sensitivity during shortening and 2) the ratio of relengthening velocity to \(k_{\text{fall}}\) to indicate sensitivity during relengthening (29). These ratios were calculated from values that were acquired simultaneously from those cardiomyocytes loaded with fura-2.

As illustrated in Fig. 5A and indicated by the statistically significant blood pressure \(\times\) training \(\times\) frequency interaction, the frequency response for the mechanical sensitivity during shortening was increased by run training in the Nt group and decreased by run training in the Ht. As depicted similarly in Fig. 5B, the frequency response for the mechanical sensitivity during relengthening was also increased by run training in the Nt group and decreased in the Ht. On the basis of these results it would be expected that the contractile apparatus and/or the passive mechanical properties of cardiomyocyte are normally influenced by run training in such a way as to induce an increased negative frequency response of shortening and relengthening under these laboratory conditions independent of the influence on \([\text{Ca}^{2+}]_i\) dynamics. Run training during mild renal hypertension, however, induced a decreased negative frequency response of the contractile apparatus and/or the passive mechanical properties of cardiomyocyte.

DISCUSSION

It has been well established that the frequency responses for cardiac contraction and relaxation are strongly influenced by \([\text{Ca}^{2+}]_i\) dynamics and the modification of \(\text{Ca}^{2+}\) regulatory proteins by protein kinase signaling pathways (1, 6, 13, 25). We found in the present study that the frequency responses for rat cardiomyocyte shortening and relengthening dynamics were differentially affected by treadmill run training and mild renal hypertension in a manner that was not mimicked by the frequency responses for \([\text{Ca}^{2+}]_i\) dynamics. Our results therefore suggest that the cardiomyocyte contractile apparatus and/or passive mechanical properties, which dictate shortening and relengthening dynamics in response to \([\text{Ca}^{2+}]_i\) dynamics, play considerable roles in determining mechanical frequency responses at the cardiomyocyte level. Furthermore, our results suggest that run training significantly modified the frequency response of the cardiomyocyte contractile apparatus and/or passive mechanical properties and that mild renal hypertension altered these effects of run training.

The specific \(\text{Ca}^{2+}\) regulatory mechanisms underlying the negative frequency response for cardiomyocyte contractile functions were not elucidated in the present study but would be expected to include SR \(\text{Ca}^{2+}\) load (4–6), which has been shown to possess a negative frequency response in normotensive and hypertensive rat cardiomyocytes examined under similar experimental conditions (30). A negative frequency response for SR \(\text{Ca}^{2+}\) load is compatible with the negative frequency responses observed in the present study for \(R_{\text{air}}\) and \(R_{\text{int}}\). However, a negative frequency response for SR \(\text{Ca}^{2+}\) load would seemingly be incompatible with the positive frequency response for \(k_{\text{fall}}\), which reflects the sum of the rates of SERCA2 and NCX1. We would therefore expect a positive frequency response for \(\text{Ca}^{2+}\) efflux by NCX1 as suggested previously (19).

The underlying \(\text{Ca}^{2+}\) regulatory mechanisms, although certainly responsible for the negative frequency
responses of \( R_{\text{eff}} \) and \( R_{\text{int}} \), and for the positive frequency response of \( k_{\text{fall}} \), could not have been fully responsible for the negative frequency responses of cardiomyocyte shortening and relengthening dynamics observed for the various experimental groups in the present study. As shown in Fig. 2A, run training in the Nt group amplified the negative frequency response for cardiomyocyte peak shortening, which could be due to 1) the amplified negative frequency response for the integral of \( [\text{Ca}^{2+}] \) transient shown in Fig. 4B but may also be due to 2) the amplified negative frequency response for \( \text{Ca}^{2+} \) sensitivity of the contractile apparatus as shown in Fig. 5A. Run training has been shown previously to increase the basal \( \text{Ca}^{2+} \) sensitivity of the contractile apparatus (10, 33). Run training in the Ht group (1) did not affect the negative frequency response for cardiomyocyte peak shortening (Fig. 2A) and yet 2) induced an amplified negative frequency response for the integral of \( [\text{Ca}^{2+}] \) transient (Fig. 4B). Therefore, as illustrated in Fig. 5A, the state of mild renal hypertension must have diminished or altered the run training effect on the frequency response for the contractile apparatus and/or other passive mechanical properties, which cause the cardiomyocyte to shorten in response to \( [\text{Ca}^{2+}] \).

The influence of mild renal hypertension on the effect of run training was also demonstrated in diastolic function. Run training in the Nt group amplified the negative frequency response for relengthening velocity (Fig. 2D) but did not affect the positive frequency response for \( k_{\text{fall}} \) (Fig. 4D). In contrast, run training in the Ht group did not appreciably affect the frequency response for relengthening velocity (Fig. 2D) but amplified a positive frequency response for \( k_{\text{fall}} \) (Fig. 4D). Therefore, as illustrated in Fig. 5E, run training in the Nt and Ht groups must have differentially affected the frequency responses for cardiomyocyte \( \text{Ca}^{2+} \) sensitivity, stiffness, viscosity, and/or actomyosin cross-bridge off-rate, which is dependent on the cardiac myosin heavy chain (MHC) isoform and ATP availability at the myofibrils.

Of the above possibilities, we believe we can rule out the increase in \( \text{Ca}^{2+} \) sensitivity and changes in MHC isoform as underlying the differential effects of run training in the Nt and Ht groups. For example, we observed a shorter time to peak shortening relative to time to peak R induced by run training in both the Nt and Ht groups, which suggests an increase in \( \text{Ca}^{2+} \) sensitivity induced by run training in both groups (10). In addition, cardiac MHC isoform, which affects actomyosin cross-bridge off-rate, may be expected to switch from \( \alpha \)-MHC to \( \beta \)-MHC in the Ht group (22). However, MHC isoform does not change with run training in the male Fischer 344 rat possessing a high density of \( \beta \)-MHC (11, 26). Therefore, the differential effects of run training on the frequency response of cardiomyocyte mechanics are not likely due to changes in myofilament \( \text{Ca}^{2+} \) sensitivity or MHC isoform but are likely due to changes in stiffness (34), viscosity, and ATP availability.

There are specific attributes of the renal hypertension model used in the present study that better put our findings into context of other rat models of hypertension. As reported from previous studies, cardiomyocytes isolated from rats with severe hypertension, which has been characterized by increased systolic blood pressure of \( >50 \) mmHg, cardiac hypertrophy, and increased cardiomyocyte width (8, 20), exhibit diminished shortening (22, 35) and \( [\text{Ca}^{2+}] \) dynamics (22). Cardiomyocytes from our model of mild renal hypertension, which was characterized by increased systolic blood pressure of 20–30 mmHg leading to no hypertrophy (16, 20) and no increase in cardiomyocyte width (24), did not exhibit any tendencies toward diminished shortening nor \( [\text{Ca}^{2+}] \) dynamics under these laboratory conditions. Instead, mild renal hypertension may have subtly increased contractile function, as best illustrated by the reduced time to peak shortening (Figs. 1 and 2B) and reduced time to peak R (Figs. 3 and 4C) in the Ht+Sed compared with Nt+Sed rats. Cardiomyocytes from our model of mild renal hypertension therefore demonstrated compensated or otherwise uncompromised function and provided a useful context, within which the combined effects of run training and renal hypertension on cardiomyocyte frequency responses could be interpreted without the complications of diminished basal function.

In conclusion, our data strongly suggest that mild renal hypertension altered the modifications to cardiomyocyte properties that would otherwise be normally induced by run training. However, the present study does not clarify which modifications in cardiomyocyte mechanical properties were affected by run training and altered by mild renal hypertension. On the basis of our results, further study of the effects of run training and renal hypertension on the contractile apparatus and passive mechanical properties of the myocardium is warranted and would likely include measures of the frequency dependence of the passive mechanical properties of \( \text{Ca}^{2+} \)-activated myofilaments.

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DISCLOSURES

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