Effects of sprint training on contractility and 
$[\text{Ca}^{2+}]_i$ transients in adult rat myocytes

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Zhang, Xue-Qian, Jianliang Song, Lois L. Carl, Weixing Shi, Anwer Qureshi, Qiang Tian, and Joseph Y. Cheung. Effects of sprint training on contractility and $[\text{Ca}^{2+}]_i$ transients in adult rat myocytes. J Appl Physiol 93: 1310–1317, 2002. First published July 5, 2002; 10.1152/japplphysiol.01071.2001.—The effects of 6–8 wk of high-intensity sprint training (HIST) on rat myocyte contractility and intracellular Ca$^{2+}$ concentration ($[\text{Ca}^{2+}]_i$) transients were investigated. Compared with sedentary (Sed) myocytes, HIST induced a modest (5%) but significant ($P < 0.0005$) increase in cell length with no changes in cell width. In addition, the percentage of myosin heavy chain α-isoenzyme increased significantly ($P < 0.02$) from 0.566 ± 0.077% in Sed rats to 0.871 ± 0.006% in HIST rats. At all three (0.6, 1.8, and 5 mM) extracellular Ca$^{2+}$ concentrations ($[\text{Ca}^{2+}]_o$) examined, maximal shortening amplitudes and maximal shortening velocities were significantly ($P < 0.0001$) lower and half-times of relaxation were significantly ($P < 0.005$) longer in HIST myocytes. HIST myocytes had significantly ($P < 0.0001$) higher diastolic $[\text{Ca}^{2+}]_i$ levels. Compared with Sed myocytes, systolic $[\text{Ca}^{2+}]_i$ levels in HIST myocytes were higher at 0.6 mM $[\text{Ca}^{2+}]_o$, similar at 1.8 mM $[\text{Ca}^{2+}]_o$, and lower at 5 mM $[\text{Ca}^{2+}]_o$. The amplitudes of $[\text{Ca}^{2+}]_i$ transients were significantly ($P < 0.0001$) lower in HIST myocytes. Half-times of $[\text{Ca}^{2+}]_i$ transient decline, an estimate of sarcoplasmic reticulum (SR) Ca$^{2+}$ uptake activity, were not different between Sed and HIST myocytes. Compared with Sed hearts, Western blots demonstrated a significant ($P < 0.03$) threefold decrease in Na$^+/\text{Ca}^{2+}$ exchanger, but SR Ca$^{2+}$-ATPase and calasequestrin protein levels were unchanged in HIST hearts. We conclude that HIST effected diminished myocyte contractile function and $[\text{Ca}^{2+}]_i$ transient amplitudes under the conditions studied. We speculate that downregulation of Na$^+/\text{Ca}^{2+}$ exchanger may partly account for the decreased contractility in HIST myocytes.

excitation-contraction coupling; cardiac hypertrophy; edge detection; fura 2; microfluorimetry; intracellular calcium concentration

HIGH-INTENSITY SPRINT TRAINING (HIST) has been shown to exert beneficial effects on rat hearts recovering from myocardial infarction (MI), both in vivo (13) and in vitro (29, 30, 32, 34). Specifically, 6 wk of HIST instituted 3 wk after MI was able to increase maximal stroke volume ($SV_{max}$) (13), reverse myocyte hypertrophy associated with MI (29, 32, 34), shorten the prolonged action potential duration by enhancing transient outward current (34), correct abnormal intracellular Ca$^{2+}$ concentration ($[\text{Ca}^{2+}]_i$) transient (30) and cell shortening dynamics (29), improve Na$^+/\text{Ca}^{2+}$ exchange (32) and sarcoplasmic reticulum (SR) Ca$^{2+}$ uptake activity (30), and shift the myosin heavy chain (MHC) isoenzyme distribution pattern back toward normal (32). In this regard, HIST is different from chronic endurance treadmill running in that, although both training regimens produced a number of beneficial effects on hemodynamics in MI rats, chronic endurance running did not result in an increase in $SV_{max}$ that could be generated by the MI rat during exercise (14, 15).

Although recent studies have provided important information on cellular and molecular adaptations in normal hearts subjected to a period of chronic endurance running (4, 5, 7–12, 16, 17, 19, 23, 25–27), there is no information on the effects of HIST on normal myocyte function despite its salutary effects on rat MI hearts discussed above. The present study was undertaken to test the hypothesis that HIST improves contractile function in single myocytes isolated from normal rat hearts.

METHODS

Exercise-training protocol. Male Sprague-Dawley rats were randomly divided into two groups: sedentary (Sed; $n = 18$) and HIST ($n = 24$). All rats received rat chow and water ad libitum and were maintained on a 12:12-h light-dark cycle. Sed rats walked on the treadmill (0° grade, 10 m/min, 10 min/day, Mondays and Thursdays) for 7–8 wk before hearts were excised for myocyte isolation. For HIST rats, after they acclimatized to the treadmill (0° grade, 10 m/min, 10 min/day, 5 days/wk) for 1 wk, the training protocol consisted of 5 consecutive 1-min running bouts daily, 5 days/wk, and each running bout was interspersed with 90 s of rest. During the first week of training, treadmill speed was set at 66 m/min and grade was set at 15°. During the second week of training, treadmill speed was progressively increased to 97 m/min. The treadmill grade and speed were then held con-

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stant for the remaining 5–7 wk of the training period (total: 6–8 wk).

Myocyte isolation and shortening measurements. Cardiac myocytes were isolated from the septum and left ventricular (LV) free wall by successive perfusion with collagenase and hyaluronidase (2). Freshly isolated myocytes were seeded on laminin-coated coverslips (3) and used within 2–6 h of isolation for contractility measurements. Briefly, myocytes adherent to coverslips were bathed in 0.6 mL of air-and-temperature-equilibrated (37°C), HEPES-buffered (20 mM, pH 7.4) medium (Earle’s balanced salt solution without L-glutamine and NaHCO_3). NaHCO_3 (25 mM) was added to medium 199, and extracellular Ca^{2+} concentration ([Ca^{2+}]_o) was adjusted to either 0.6, 1.8, or 5.0 mM. Coverslips containing myocytes were placed on a temperature-controlled (37°C) stage of a Zeiss IM35 microscope (29, 33). Fields of myocytes were chosen at random, and myocytes were field stimulated to contract (1 Hz) between platinum electrodes spaced 2 mm apart, as previously described (29, 30, 33). Myocytes viewed through an Olympus DApouV ×40/1.30 numerical aperature oil objective were imaged by a charge-coupled device video camera (Ionoptix, Milton, MA). Myocyte lengths, widths, and motion measurements were acquired by a personal computer with interface and software purchased from Ionoptix. Data were permanently stored on a zip drive (Iomega, Roy, UT) and analyzed off-line by Ionoptix software. For calibration of pixels vs. micrometers, a high-resolution coupled device video camera (Ionoptix, Milton, MA) was used.

In general, 4–10 myocytes on each coverslip were studied within 30 min. Each myocyte contraction measurements lasted 30 s. Medium on the coverslip was completely exchanged four to six times during the 30-min experimental period. Under these conditions, there was no run down, i.e., when stimulated in sequential 30-s periods, myocytes continued to beat with similar steady-state amplitudes at the imposed pacing frequency. Myocytes on each coverslip were only studied at one [Ca^{2+}]_o.

[Ca^{2+}]_o transient measurements. Myocytes were exposed to 0.67 μM fura 2-AM for 15 min at 37°C (3). Fura 2-loaded myocytes mounted in a Dvorak-Stotler chamber situated in a temperature-controlled stage (37°C) of a Zeiss IM35 inverted microscope were field stimulated to contract at 1 Hz between platinum wire electrodes, as previously described (30, 31, 33). [Ca^{2+}]_o was varied between 0.6 and 5.0 mM. Excitation light (360 and 380 nm, ±10-nm band pass, Ionoptix) was directed to individual myocytes only during data acquisition to minimize photobleaching. Epifluorescence (510 ± 18 nm) was collected by an Olympus DApou UV ×40/1.30 numerical aperature oil objective was passed through a pinhole (1.6 mm) and captured by a photomultiplier (model R928-07, Hamamatsu). Output from the photomultiplier was routed through an amplifier/discriminator (model C609, Thorn EMI, Middlesex, UK) before arrival at a counter/timer board (model C660, Thorn EMI).

Epifluorescence from a myocyte collected at 360-nm excitation was divided by that collected at 380-nm excitation to obtain the fluorescence intensity ratio (R), from which [Ca^{2+}]_o was calculated by using 224 nM as the Ca^{2+}-fura 2 dissociation constant (3, 30, 31, 33). Background and cellular autofluorescence measured in myocytes not loaded with fura 2 accounted for <5% of the fura 2 signal. Intraacellular fura 2 fluorescence was calibrated daily for each batch of myocytes, as previously described (3). [Ca^{2+}]_o transient data were analyzed with custom-written software (Ionoptix).

Na^+ /Ca^{2+} exchanger, sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA2), and calsequestrin immunoblotting, and MHC isoenzyme pattern determination. Pieces of LV and septum from Sed and HIST rats were homogenized in 2 mL of ice-cold lysis buffer containing (in mM) 50 Tris (pH 8.0), 150 NaCl, 100 sodium fluoride, 1 EDTA, 1 EGTA, 1 phenylmethylsulfonyl fluoride, 1 sodium orthovanadate, 0.5% Nonidet P-40, 10 μg/mL leupeptin, and 10 μg/mL aprotinin. The tissue homogenates were snap frozen with dry ice-ethanol and stored at −80°C.

Heart homogenates in SDS sample buffer (containing either 10 mM N-ethylmaleimide [for Na^+ /Ca^{2+} exchanger (NCX1)] or 5% 2-mercaptoethanol [for sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA2)]) were applied to 7.5% polyacrylamide gel, and proteins were separated by electrophoresis (30–33). Proteins from SDS-polyacrylamide gel electrophoresis were transferred onto Immuno-Blot polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA). To detect NCX1, rabbit anti-NCX1 antibody (1:500 dilution; π11–13, Swant; Bellinzona, Switzerland) was used with donkey anti-rabbit IgG (1:5,000; Amersham, Buckinghamshire, UK) as the secondary antibody. SERCA2 was detected with a monoclonal antibody (1:1,000; MA3-919, Affinity Bioreagents, Golden, CO), and sheep anti-mouse antibody (1:2,000; Amersham) was used as the secondary antibody. For calsequestrin immunoblotting, membranes stripped of NCX1 or SERCA2 antibodies were sequentially exposed to rabbit anti-calsequestrin antibody (1:2,500; Swant) and donkey anti-rabbit IgG (1:5,000; Amersham). Our laboratory has previously used π11–13, MA3-919, and anti-calsequestrin antibodies to successfully detect NCX1, SERCA2, and calsequestrin, respectively (30–33). Immunoreactive proteins were detected with the enhanced chemiluminescence-Western blotting system (Amersham). Protein band signal intensities were quantitated by scanning autoradiograms of the blots with a phosphoimager (Molecular Dynamics, Sunnyvale, CA).

For analysis of MHC isoenzyme distributions, heart homogenates (1 μg/lane) were subjected to SDS-polyacrylamide gel (5%) electrophoresis. Silver stain was used to visualize MHC α- and β-isoenzymes as previously described (32).

Statistics. All results are expressed as means ± SE. In experiments in which maximal contraction and [Ca^{2+}]_o transient amplitudes were measured as functions of experimental groups (Sed vs. HIST) and [Ca^{2+}]_o, two-way ANOVA was performed to determine significance of difference. A linear model fitted standard least squares (JMP version 4, SAS Institutes, Cary, NC) was used. Single between-group comparisons (e.g., cell lengths and widths, NCX1 abundance) were made by unpaired Student’s t-tests. In all analysis, P ≤ 0.05 was taken to be statistically significant.

RESULTS

Effects of HIST on myocyte size and myosin isoenzyme distribution. After 6–8 wk of HIST, LV myocytes isolated from HIST hearts averaged 138.5 ± 2.2 μm (n = 100 myocytes from 3 rats) and were significantly (P < 0.0005) longer than those isolated from Sed hearts (131.9 ± 4.2 μm, n = 72 myocytes from 3 rats). There were no differences (P > 0.08) in cell widths between Sed (26.9 ± 0.4 μm) and HIST (26.0 ± 0.4 μm) myocytes. The ~5% increase in cell length in HIST myocytes in the present paper was similar to that observed in cardiac myocytes isolated from rats subjected to 20–30 wk of chronic endurance treadmill training (11, 1311EXERCISE TRAINING AND CARDIAC MYOCYTE SHORTENING

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19). In addition, 8 wk (n = 3 rats) but not 2 wk (n = 3 rats) of HIST effected a significant (P < 0.0207) increase in relative MHC α-isoenzyme abundance (0.871 ± 0.006%) compared with Sed hearts (0.566 ± 0.077%, n = 4 rats; Fig. 1), attesting to the efficacy of our exercise training regimen.

**Effects of HIST on myocyte contractile function.** At all three [Ca\(^{2+}\)]\(_o\) examined, HIST myocytes (n = 5 rats) shortened significantly (P < 0.0001) less than Sed myocytes (n = 4 rats) (Fig. 2 and Table 1). In addition, the differences in maximal contraction amplitudes between Sed and HIST myocytes were amplified as


Table 1. Effects of HIST on myocyte shortening dynamics

<table>
<thead>
<tr>
<th>[Ca(^{2+})](_o)</th>
<th>Sed</th>
<th>HIST</th>
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</thead>
<tbody>
<tr>
<td>Maximal contraction amplitude, % resting cell length</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td>5.68 ± 0.47 (29)</td>
<td>5.32 ± 0.48 (33)*</td>
</tr>
<tr>
<td>1.8</td>
<td>14.26 ± 0.50 (38)</td>
<td>11.87 ± 0.39 (40)*</td>
</tr>
<tr>
<td>5.0</td>
<td>17.70 ± 0.35 (25)</td>
<td>14.68 ± 0.40 (37)*</td>
</tr>
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</table>

| Maximal shortening velocity, cell length/s |     |     |
| 0.6 | 0.57 ± 0.06 | 0.67 ± 0.05* |
| 1.8 | 1.62 ± 0.09 | 1.38 ± 0.06* |
| 5.0 | 2.15 ± 0.11 | 1.69 ± 0.07* |

| \(t_{1/2}\), ms |     |     |
| 0.6 | 75.1 ± 4.8 | 92.0 ± 6.8† |
| 1.8 | 87.1 ± 3.6 | 96.9 ± 4.5† |
| 5.0 | 80.7 ± 3.2 | 98.1 ± 4.9† |

Values are means ± SE. Nos. in parentheses are no. of myocytes, without regard to the no. of cells contributed by each heart. [Ca\(^{2+}\)]\(_o\), extracellular Ca\(^{2+}\) concentration; HIST, high-intensity sprint training (n = 5 rats); Sed, sedentary (n = 4 rats); \(t_{1/2}\), half-time of relaxation. *P < 0.0001, †P < 0.005, Sed vs. HIST.

[Ca\(^{2+}\)]\(_o\) was increased. These conclusions are supported by the results of two-way ANOVA, which indicates significant group (P < 0.0001), [Ca\(^{2+}\)]\(_o\) (P < 0.0001), and group × [Ca\(^{2+}\)]\(_o\) interaction (P < 0.04) effects.

To further analyze contraction dynamics, we measured maximal shortening velocity and half-time of relaxation (\(t_{1/2}\)) (Table 1). Maximal shortening velocity was significantly (P < 0.0001, group effect) lower in HIST myocytes. Raising [Ca\(^{2+}\)]\(_o\) increased maximal shortening velocity (significant [Ca\(^{2+}\)]\(_o\) effect, P < 0.0001), but it did not affect the inherent differences in maximal shortening velocities between Sed and HIST myocytes (group × [Ca\(^{2+}\)]\(_o\) effect, P > 0.3).

Compared with Sed myocytes, HIST myocytes had similarly prolonged \(t_{1/2}\) (Table 1; significant group effect, P < 0.005) across all three [Ca\(^{2+}\)]\(_o\) examined (insignificant group × [Ca\(^{2+}\)]\(_o\) effect, P > 0.4). Surprisingly, increasing [Ca\(^{2+}\)]\(_o\) had no effect on \(t_{1/2}\) ([Ca\(^{2+}\)]\(_o\) effect, P > 0.8).

Effects of HIST on [Ca\(^{2+}\)]\(_i\) transients. [Ca\(^{2+}\)]\(_i\) occupies a central role in cardiac myocyte excitation-contraction coupling. Thus the differences in contractile behavior between Sed and HIST myocytes may be related to differences in [Ca\(^{2+}\)]\(_i\), homeostasis brought about by 8 wk of HIST. Indeed, end-diastolic [Ca\(^{2+}\)]\(_i\) levels were significantly higher in myocytes isolated from four HIST hearts compared with myocytes isolated from three Sed hearts (Fig. 3 and Table 2; group effect, P < 0.0001). Changing [Ca\(^{2+}\)]\(_o\) had no significant effect on diastolic [Ca\(^{2+}\)]\(_i\) levels (Table 2; [Ca\(^{2+}\)]\(_o\) effect, P > 0.7). Elevated diastolic [Ca\(^{2+}\)]\(_i\) levels in HIST myocytes suggested that Na\(^+\)/Ca\(^{2+}\) exchange and/or SR Ca\(^{2+}\)-ATPase activities were depressed (28).

With respect to systolic [Ca\(^{2+}\)]\(_i\), raising [Ca\(^{2+}\)]\(_o\) increased systolic [Ca\(^{2+}\)]\(_i\) in both Sed and HIST myocytes (Table 2; [Ca\(^{2+}\)]\(_o\) effect, P < 0.0001). At low [Ca\(^{2+}\)]\(_o\) (0.6 mM), systolic [Ca\(^{2+}\)]\(_i\) values for HIST myocytes were higher than those found for Sed myocytes (Fig. 3 and Table 2). In contrast, at high [Ca\(^{2+}\)]\(_o\) (5 mM), Sed myocytes had higher systolic [Ca\(^{2+}\)]\(_i\) values (Fig. 3 and Table 2). At intermediate [Ca\(^{2+}\)]\(_o\) (1.8 mM), differences in systolic [Ca\(^{2+}\)]\(_i\) between Sed and HIST myocytes were narrowed. This interpretation is supported by the results of two-way ANOVA: insignificant group (P > 0.5) but significant group × [Ca\(^{2+}\)]\(_o\) interaction (P = 0.0006) effects, indicating that the magnitude and/or direction of the effects of [Ca\(^{2+}\)]\(_o\) on systolic [Ca\(^{2+}\)]\(_i\) was different across Sed and HIST myocytes.

The magnitude of the [Ca\(^{2+}\)]\(_i\) transient is reflected by the percent increase in fura 2 R, which is free from fluorescence calibration errors and uncertainties in intracellular fura 2 dissociation constant. As a group, the magnitude of [Ca\(^{2+}\)]\(_i\) transient was significantly higher in Sed myocytes (Fig. 3 and Table 2; group effect, P < 0.0001). Raising [Ca\(^{2+}\)]\(_o\) increased the percent increase in R in both groups ([Ca\(^{2+}\)]\(_i\) effect, P < 0.0001), but the increase in [Ca\(^{2+}\)]\(_i\) transient magnitude with elevating [Ca\(^{2+}\)]\(_o\) was larger in Sed than in HIST myocytes (group × [Ca\(^{2+}\)]\(_o\) interaction effect, P < 0.0001).

Comparisons of \(t_{1/2}\) of [Ca\(^{2+}\)]\(_i\) transient decline indicated no significant differences between HIST and Sed myocytes (Table 2; group effect, P > 0.2). Because \(t_{1/2}\) of [Ca\(^{2+}\)]\(_i\) transient decline was a reasonable in vivo estimate of SR Ca\(^{2+}\) uptake (28, 31), our observation suggests that SR Ca\(^{2+}\) uptake activities were similar between HIST and Sed myocytes. Elevating [Ca\(^{2+}\)]\(_o\), which increased the amplitudes of [Ca\(^{2+}\)]\(_i\) transients, significantly lowers the \(t_{1/2}\) of [Ca\(^{2+}\)]\(_i\); decline in both Sed and HIST myocytes (Table 2; [Ca\(^{2+}\)]\(_o\) effect, P < 0.0001), consistent with the report by Bers and Berlin (1) that the kinetics of [Ca\(^{2+}\)]\(_i\) decline were dependent on peak [Ca\(^{2+}\)]\(_o\).

Effects of HIST on NCX1, SERCA2, and calsequestrin abundance in rat hearts. Results from [Ca\(^{2+}\)]\(_i\) transient measurements (elevated diastolic [Ca\(^{2+}\)]\(_i\) levels and similar \(t_{1/2}\) of [Ca\(^{2+}\)]\(_i\); decline) suggest that Na\(^+\)/Ca\(^{2+}\) exchange but not SR Ca\(^{2+}\) uptake was depressed in HIST myocytes. An independent approach to support this interpretation was to measure NCX1, SERCA2, and calsequestrin protein levels in LV and septum of Sed and HIST rats. Under nonreducing gel conditions, NCX1 was detected as a band of apparent molecular mass of 160 kDa (20, 32, 33). Compared with Sed hearts (n = 4), HIST hearts (n = 6) had significantly (P < 0.027) less NCX1 protein (1,158 ± 280 vs. 3,459 ± 981 arbitrary units) (Fig. 4). By contrast, there were no (P = 0.14) differences in SERCA2 amounts between Sed (7,239 ± 539) and HIST (5,203 ± 939 arbitrary units) hearts (Fig. 4). Similarly, calsequestrin abundance was similar between Sed (3,678 ± 491 arbitrary units) and HIST (2,017 ± 615 arbitrary units) hearts (P = 0.09) (Fig. 4).

DISCUSSION

Exercise training has been shown to have salutary effects on cardiac performance in both normal and diseased hearts (for review, see Ref. 12). Despite the wealth of information on the effects of exercise training
on heart function in vivo and in vitro, to date there are only five published reports that examined the effects of exercise training on isolated myocyte shortening dynamics (9, 11, 16, 19, 27). In the four studies that utilized endurance treadmill running, training was shown to effect an increase (11, 27), decrease (19), or no change (9) in maximal shortening amplitudes. In a recent study employing a voluntary exercise (running wheel) model, exercise training had no effect on cell shortening (16). These differences may well relate to different experimental conditions (pacing frequency, [Ca$^{2+}]_o$, temperature) (9, 11, 19), exercise regimens, or whether cell shortening was measured in myocytes exposed to DMSO (19) and/or loaded with the Ca$^{2+}$ indicator fura 2 (9, 19).

In normal rat myocardium, both chronic endurance running (7, 12) and HIST (6) increased maximal cardiac output by increasing $SV_{max}$. In the postinfarction myocardium, only HIST (13), but not chronic endurance running (14, 15), effected a statistically significant increase in $SV_{max}$. At least part of the beneficial effects of HIST on cardiac contractility in the postinfarction LV was due to enhanced contractility at the single myocyte level (29), plausibly due to improvement by HIST in the many Ca$^{2+}$ homeostatic pathways involved in excitation-contraction coupling (30, 32, 34). There is at present no information on the effects of HIST on contractile function of myocytes isolated from normal rat hearts.

In the present study, HIST elicited increases in LV myocyte length (~5%) but no changes in cell widths. This finding is similar to the modest myocyte hypertrophy (also ~5% increase in cell length with no differences in cell widths) observed in chronic endurance running rats (10, 11, 19). In addition, the expression of $\alpha$-MHC was enhanced by HIST (Fig. 1), in agreement with the ~30% increase in $\alpha$-MHC mRNA after 13 wk of chronic treadmill running (7). These two cellular

Fig. 3. HIST alters intracellular Ca$^{2+}$ concentration transients in rat myocytes. Fura 2-loaded myocytes were paced (1 Hz) to contract at 37°C and a [Ca$^{2+}]_o$ of 0.6 (A and B), 1.8 (C and D), and 5.0 mM (E and F). Note that end-diastolic intracellular Ca$^{2+}$ concentration levels were higher in HIST (B, D, and F) compared with Sed (A, C, and E) myocytes. Amplitudes of intracellular Ca$^{2+}$ concentration transients were higher in Sed myocytes. Composite results are summarized in Table 2.
early studies involving treadmill-trained female rats demonstrated a decrease in $K_m$ for Ca$^{2+}$ but no change in $V_{max}$ in Na$^+$/Ca$^{2+}$ exchange uptake in highly purified sarcosomal (SL) vesicles (26). Subsequently, Na$^+$/Ca$^{2+}$ exchange activity in SL vesicles was shown to be unaffected by training in swimming male rats (21) and running pigs (8). In female rats subjected to endurance treadmill running, Ca$^{2+}$ efflux mediated by Na$^+$/Ca$^{2+}$ exchange was decreased in isolated cardiac myocytes (17), but NCX1 abundance was unchanged (27). By contrast, chronic treadmill running in male rats significantly enhanced Na$^+$/Ca$^{2+}$ efflux during caffeine-induced SR Ca$^{2+}$ release (18). The reasons for the discrepancies in results reported by different investigators on the effects of exercise training on Na$^+$/Ca$^{2+}$ exchange are not clear but may relate to different exercise regimens (chronic endurance running vs. swimming vs. HIST), assaying techniques (Na$^+$/Ca$^{2+}$ dependent uptake in SL vesicles vs. immunoblotting vs. relaxation from caffeine-induced contracture in intact myocytes), sex (male vs. female), strain (Sprague-Dawley vs. Fischer), and species (rat vs. pig) differences. Despite the controversies concerning exercise training and cardiac NCX1 activity and abundance, our present observation that HIST decreased cardiac myocyte contractile amplitude can be partly explained by decreases in NCX1 activity and abundance, and it is in agreement with more recent reports by Palmer et al. (17, 19), who used chronic endurance running as the exercise-training regimen.

The third major finding of this study is that the magnitudes of [Ca$^{2+}$]$_i$ transients were lower in HIST myocytes (Fig. 3 and Table 2). The decrease [Ca$^{2+}$]$_i$ levels suggested that Na$^+$-$\text{Ca}^{2+}$ exchange during de

markers of the “trained” state validate the efficacy of the HIST protocol used in this study.

The first major finding of the present study is that HIST exerted significant decreases in maximal contraction amplitude and maximal shortening velocity, as well as slowing of relaxation in myocytes isolated from normal rat hearts (Fig. 2 and Table 1). In cardiac myocytes isolated from rats subjected to $\geq$20 wk of chronic endurance running, Palmer et al. (19) also reported statistically significant decreases in peak cell shortening at 0.75 and 2.0 mM [Ca$^{2+}$]$_o$. The cellular mechanisms by which HIST depressed myocyte contractility are at present unknown.

As a first approach to dissect some of the mechanisms by which HIST reduced contractility in myocytes isolated from normal rat hearts, we compared [Ca$^{2+}$]$_i$, intracellular Ca$^{2+}$ concentration. HIST, n = 4 rats; Sed, n = 3 rats. $^*P < 0.001$, $^\dagger P < 0.001$, Sed vs. HIST.

Table 2. Effects of HIST on myocyte [Ca$^{2+}$]$_i$ transients

<table>
<thead>
<tr>
<th>[Ca$^{2+}$]$_o$</th>
<th>Sed</th>
<th>HIST</th>
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<tr>
<td>Diastolic [Ca$^{2+}$]$_i$, nM</td>
<td></td>
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<tr>
<td>0.6</td>
<td>106 ± 5 (51)</td>
<td>138 ± 11 (54) $^*$</td>
</tr>
<tr>
<td>1.8</td>
<td>109 ± 5 (52)</td>
<td>144 ± 6 (109) $^*$</td>
</tr>
<tr>
<td>5.0</td>
<td>104 ± 6 (49)</td>
<td>148 ± 5 (94) $^*$</td>
</tr>
<tr>
<td>Systolic [Ca$^{2+}$]$_i$, nM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td>161 ± 5</td>
<td>226 ± 15$^\dagger$</td>
</tr>
<tr>
<td>1.8</td>
<td>300 ± 18</td>
<td>308 ± 10</td>
</tr>
<tr>
<td>5.0</td>
<td>406 ± 20</td>
<td>357 ± 12$^\dagger$</td>
</tr>
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| Increase in fluorescence intensity ratio, % |      |      |
| 0.6            | 7.5 ± 0.6    | 10.1 ± 0.5$^*$ |
| 1.8            | 20.5 ± 1.6   | 18.4 ± 0.8$^*$ |
| 5.0            | 34.4 ± 1.5   | 23.0 ± 1.1$^*$ |
| $t_{1/2}$ of [Ca$^{2+}$]$_i$ transient decline, ms |      |      |
| 0.6            | 224 ± 12     | 193 ± 10 |
| 1.8            | 187 ± 7      | 175 ± 7 |
| 5.0            | 138 ± 5      | 157 ± 5 |

Values are means ± SE. Nos. in parentheses are no. of myocytes, with regard to the no. of cells contributed by each heart. [Ca$^{2+}$]$_i$, intracellular Ca$^{2+}$ concentration. HIST, n = 4 rats; Sed, n = 3 rats. $^*P < 0.0001$, $^\dagger P < 0.001$, Sed vs. HIST.

Fig. 4. HIST decreases Na$^+$/Ca$^{2+}$ exchanger (NCX1) but not sarcoplasmatic reticulum Ca$^{2+}$-ATPase (SERCA2) and calsequestrin in cardiac myocytes. Proteins in heart homogenates (50 μg/lane) were separated by gel electrophoresis and transferred to Immun-Blot polyanlyden difluoride membranes. NCX1, SERCA2, and calsequestrin were identified by immunoblotting, as described in METHODS. Note that compared with Sed hearts (n = 4), HIST hearts (n = 6) had significant decreases in NCX1. There were no differences in either SERCA2 or calsequestrin expression between Sed and HIST hearts. Composite results are presented in RESULTS. Numbers at left are apparent molecular mass.
transient amplitudes agreed well with the lower contractility in HIST myocytes (Fig. 2 and Table 1) and suggested that decreases in Ca\(^{2+}\) sensitivity of myofilaments needed not be invoked to account for diminished cell shortening. Indeed, other exercise-training paradigms such as chronic treadmill running are known to either increase (5, 11, 27) or have no effect (19) on Ca\(^{2+}\) sensitivity of myofibrillar ATPase. In contrast to HIST, chronic endurance running did not affect [Ca\(^{2+}\)]\(_i\) transients (9, 19).

Another potential mechanism by which HIST may reduce myocyte contraction is downregulation of SERCA2 abundance or activity. A lower SR Ca\(^{2+}\) uptake would not only decrease SR Ca\(^{2+}\) content and thus affect contraction amplitude but would also prolong relaxation \(t_{1/2}\). Thus the fourth major finding is that neither SERCA2 amounts (Fig. 4) nor SR Ca\(^{2+}\) uptake (as estimated by \(t_{1/2}\) of [Ca\(^{2+}\)]\(_i\); decline, Table 2) was affected by HIST. In this light, it is interesting to note that there is little evidence that chronic treadmill training increases SR Ca\(^{2+}\) uptake in normal hearts. In adult dogs (23), pigs (8), and rats (4, 22), chronic treadmill training did not alter SR Ca\(^{2+}\) pump activity or ventricular relaxation, although myocardial SERCA2 protein level was increased 21% in the treadmill-trained rat in one study (27) but no change in SERCA2 mRNA level was reported in another study (7). Our observation that HIST did not affect SERCA2 protein levels (Fig. 4) is in agreement with the vast majority of studies on exercise training and SR Ca\(^{2+}\)-ATPase function. However, treadmill running could certainly exert modulatory effects on SR Ca\(^{2+}\)-ATPase under pathophysiological settings (24, 30).

The decrease in single myocyte contractility in HIST myocytes seems counterintuitive in view of the well-documented enhancement in cardiac performance by exercise training. Specifically, in rat hearts in vivo, HIST resulted in significant increases in cardiac output and SV\(_{\text{max}}\) (6). The apparent discrepancy between single myocyte contractility and whole heart performance in HIST rats can be reconciled if one considers the fact that HIST induced a ~5% increase in resting cell length. In an elliptical chamber (e.g., the LV), a 5% increase in circumferential dimension (due to a 5% increase in LV myocyte length) would translate into a 16% increase in the volume of the chamber. Compared with Sed hearts, a smaller myocyte fractional shortening would be required to elicit a similar stroke volume in HIST hearts, and this may be of some energetics and regulatory economic advantage.

In summary, HIST for 6–8 wk resulted in an ~5% increase in myocyte length, but no changes in cell width, and an ~54% increase in relative MHC a-isoenzyme abundance. Myocyte contraction and [Ca\(^{2+}\)]\(_i\) transient amplitudes, maximal shortening velocity, and rate of relaxation, however, were reduced by HIST in normal rat myocytes. Compared with Sed myocytes, diastolic [Ca\(^{2+}\)]\(_i\) levels were higher, magnitudes of [Ca\(^{2+}\)]\(_i\) transients were lower, but \(t_{1/2}\) values of [Ca\(^{2+}\)]\(_i\) transient decline were similar in HIST myocytes. Western blots indicated that NCX1 but not SERCA2 and calsequestrin amounts was decreased in HIST myocytes. We speculate that downregulation of NCX1 may play an important role in the decreased contractility in HIST myocytes.

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