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# Sprint training restores normal contractility in postinfarction rat myocytes

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**Zhang, Lian-Qin, Xue-Qian Zhang, Timothy I. Musch, Russell L. Moore, and Joseph Y. Cheung.** Sprint training restores normal contractility in postinfarction rat myocytes. *J Appl Physiol* 89: 1099–1105, 2000.—The significance of 6–8 wk of high-intensity sprint training (HIST) on contractile abnormalities of myocytes isolated from rat hearts with prior myocardial infarction (MI) was investigated. Compared with the sedentary (Sed) condition, HIST attenuated myocyte hypertrophy observed post-MI primarily by reducing cell lengths but not cell widths. At high extracellular  $\text{Ca}^{2+}$  concentration (5 mM) and low pacing frequency (0.1 Hz), conditions that preferentially favored  $\text{Ca}^{2+}$  influx over efflux, MI-Sed myocytes shortened less than Sham-Sed myocytes did. HIST significantly improved contraction amplitudes in MI myocytes. Under conditions that favored  $\text{Ca}^{2+}$  efflux, i.e., low extracellular  $\text{Ca}^{2+}$  concentration (0.6 mM) and high pacing frequency (2 Hz), MI-Sed myocytes contracted more than Sham-Sed myocytes. HIST did not appreciably affect contraction amplitudes of MI myocytes under these conditions. Compared with MI-Sed myocytes, HIST myocytes showed significant improvement in time required to reach one-half maximal contraction amplitude shortening, maximal myocyte shortening and relengthening velocities, and half time of relaxation. Our results indicate that HIST instituted shortly after MI improved cellular contraction in surviving myocytes. Because our previous studies demonstrated that, in post-MI myocytes, HIST improved intracellular  $\text{Ca}^{2+}$  dynamics, enhanced sarcoplasmic reticulum  $\text{Ca}^{2+}$  uptake and  $\text{Ca}^{2+}$  content, and restored  $\text{Na}^+/\text{Ca}^{2+}$  exchange current toward normal, we hypothesized that improvement in MI myocyte contractile function by HIST was likely mediated by normalization of cellular  $\text{Ca}^{2+}$  homeostatic mechanisms.

exercise training; excitation-contraction coupling; cardiac hypertrophy; heart; systolic dysfunction; video imaging

EXERCISE TRAINING-INDUCED improvements in intrinsic cardiac contractile function in normal hearts have been well established (22, 27, 28). When instituted after myocardial infarction (MI), exercise training has also been shown to exert beneficial effects on cardiovascular function in both humans (11, 13) and animals (23, 24). Focusing on cellular mechanisms that may contribute

to exercise training-induced enhancements in cardiac function, our laboratory has recently shown in rats that a program of high-intensity sprint training (HIST) instituted shortly after MI was effective in attenuating myocyte hypertrophy, restoring myosin heavy chain isoenzyme distribution toward normal, increasing  $\text{Na}^+/\text{Ca}^{2+}$  currents ( $I_{\text{Na}/\text{Ca}}$ ), normalizing intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) transients during excitation-contraction, improving sarcoplasmic reticulum (SR)  $\text{Ca}^{2+}$  uptake, and enhancing SR  $\text{Ca}^{2+}$  content (30, 35).

At the level of a single cell, surviving myocytes isolated from hearts post-MI demonstrated either increase (18, 33), decrease (6, 15, 17, 20, 33), or no change (3, 6, 18, 33) in maximal myocyte contraction amplitudes. The discrepancies in results reported by different investigators may relate to differences in animal species, infarct sizes, experimental conditions [temperature, extracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_o$ ), and pacing frequency], and extent of left ventricular (LV) remodeling post-MI. In a previous study, we characterized contraction abnormalities in myocytes isolated from rat hearts 3 wk post-MI (33). Under conditions that preferentially favored  $\text{Ca}^{2+}$  efflux over influx (low  $[\text{Ca}^{2+}]_o$  and high pacing frequency), MI myocytes shortened more than those from rats that had received sham operations. Conversely, under conditions that favored  $\text{Ca}^{2+}$  influx (high  $[\text{Ca}^{2+}]_o$  and low pacing frequency), Sham myocytes shortened to a greater extent than MI myocytes. At intermediate  $[\text{Ca}^{2+}]_o$  and pacing frequencies, differences in steady-state contractile amplitudes between Sham and MI myocytes were no longer significant. These observations were consistent with the hypothesis that both  $\text{Ca}^{2+}$  influx and efflux pathways were subnormal in MI myocytes and that they contributed to abnormal myocyte contraction. Of the major  $\text{Ca}^{2+}$  influx and efflux pathways in our 3-wk rat MI model, only  $I_{\text{Na}/\text{Ca}}$  (36), but not  $\text{Ca}^{2+}$  current ( $I_{\text{Ca}}$ ; 32), was depressed in MI myocytes. Because HIST was effective in improving  $I_{\text{Na}/\text{Ca}}$  and  $[\text{Ca}^{2+}]_i$  dynamics

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in MI myocytes (30, 35), the present study was undertaken to evaluate whether HIST would restore contractile behavior of MI myocytes toward normal.

## METHODS

**Animal preparation and exercise-training protocol.** MI was induced in male Sprague-Dawley rats (~300 g) by techniques previously described in detail (6, 23, 24, 30–36). The sham operation was identical except that the coronary artery was not occluded. All surviving rats received rat chow and water ad libitum and were maintained on a 12:12-h light-dark cycle. At 2 wk postoperation, all rats were started on the treadmill (0° grade, 10 m/min, 10 min/day, 5 days/wk; Precision Biomedical Systems, State College, PA). At 3 wk postoperation, sham-operated rats ( $526 \pm 13$  g,  $n = 15$ ) continued to walk on the treadmill at low intensity (0° grade, 10 m/min, 10 min/day, Mondays and Thursdays) (Sham-Sed) for another 7–9 wk before death. At 3 wk postoperation, MI rats were randomly assigned to either a sedentary (MI-Sed;  $524 \pm 12$  g,  $n = 13$ ) or training (MI-HIST;  $492 \pm 8$  g,  $n = 13$ ) group. Animal weights were measured at time of death. MI-Sed rats participated in the same treadmill-walking program as Sham-Sed rats for 6–8 wk before they were killed. During the first week of training, MI-HIST rats ran five consecutive 1-min bouts daily, 5 days/wk, and each running bout was interspersed with 60 s of rest. Treadmill speed and grade were set at 66 m/min and 15°, respectively. During the second week of training (week 5 post-MI), treadmill speed was progressively increased to 97 m/min. The treadmill grade and speed were then held constant for the remainder of the training period.

**Myocyte isolation.** After 6–8 wk of training (9–11 wk postoperation), cardiac myocytes were isolated separately from the septum and LV free wall by methods previously described in detail (7, 8). Isolated myocytes were seeded onto laminin-coated coverslips in 2 ml of medium 199 (pH 7.4, 95% air-5% CO<sub>2</sub>, 37°C) for 2 h before contraction experiments. Myocytes were randomly chosen for experiments from among those that retained rod-shape and sharp cross striations, adhered to cover glass, and showed no membrane blebs.

**Myocyte shortening measurements.** 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 199 containing either 0.6 or 5.0 mM [Ca<sup>2+</sup>]<sub>o</sub> and were placed on a temperature-controlled stage (37°C) of a Zeiss IM35 inverted microscope (6, 29, 33). Measurements of myocyte contractile activity were made between 2 and 6 h after isolation, because overnight culture (without continuous pacing) resulted in significant slowing of cell-shortening dynamics (29). Fields of myocytes were chosen at random, and myocytes were field stimulated to contract between platinum wire electrodes spaced 2 mm apart, as previously described (6, 22, 29, 33). Stimulus conditions were either 0.6 mM [Ca<sup>2+</sup>]<sub>o</sub> and 2 Hz or 5.0 mM [Ca<sup>2+</sup>]<sub>o</sub> and 0.1 Hz. For brevity, only the value of [Ca<sup>2+</sup>]<sub>o</sub> was explicitly mentioned throughout the text, with the understanding that 0.6 mM [Ca<sup>2+</sup>]<sub>o</sub> was always paired with 2 Hz, and 5.0 mM [Ca<sup>2+</sup>]<sub>o</sub> with 0.1 Hz. These conditions were chosen to maximize the contractile differences between Sham and MI myocytes (6, 33). It should be noted that, in our rat MI model, there were no contraction amplitude differences between Sham and MI myocytes when paced at [Ca<sup>2+</sup>]<sub>o</sub> of 1.8 mM at 0.1–5 Hz (33). Myocytes viewed through an Olympus DApoUV ×40/1.30 numerical aperture oil objective were imaged by a charge-coupled device video camera (Ionoptix, Milton, MA), and myocyte length, width, and motion measurements were acquired by a personal com-

puter 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 test target (model 22–8635, Ealing Electro-Optics, Natick, MA) was used.

**Statistics.** All results are expressed as means ± SE. In analyses of a parameter (e.g., contraction amplitude) as a function of group (Sham-Sed, MI-Sed, MI-HIST), location (septum, LV free wall), and [Ca<sup>2+</sup>]<sub>o</sub> (0.6, 5.0 mM), three-way ANOVA was performed to determine significance of difference. A linear model fitted by standard least squares in a commercial software package (JMP version 3.1, SAS Institute, Cary, NC) was used. In all analyses,  $P \leq 0.05$  was taken to be statistically significant.

## RESULTS

**Effects of MI ± HIST on surviving cardiac myocyte sizes.** For all three groups, there were no differences in cell lengths or cell widths measured in myocytes isolated from the septum (distant to infarct) or LV free wall (proximal to infarct) (insignificant location and group × location interaction effects, Table 1). Two-way ANOVA indicates significant differences in mean cell lengths ( $P < 0.0001$ ) but not mean cell widths ( $P = 0.16$ ) among the three groups (Table 1). When cell length data were analyzed without regard to location (composite in Table 1), MI-Sed myocytes were significantly ( $P < 0.0001$ ) longer (by 14%) than Sham-Sed myocytes. Six to eight weeks of HIST instituted 3 wk post-MI significantly ( $P < 0.0001$ ) reduced cell length, although not back to values measured in Sham-Sed myocytes (Table 1).

**Effects of MI ± HIST on myocyte motion dynamics.** Figure 1 shows representative steady-state twitches in Sham-Sed, MI-Sed, and MI-HIST myocytes, from which maximal contraction amplitude (% resting cell

Table 1. Effects of MI and HIST on myocyte dimensions

| Location        | Sham-Sed      | MI-Sed        | MI-HIST       |
|-----------------|---------------|---------------|---------------|
| Cell length, μm |               |               |               |
| Septum          | 111 ± 2 (67)  | 133 ± 3 (40)  | 119 ± 2 (75)  |
| LV free wall    | 117 ± 3 (62)  | 128 ± 3 (52)  | 119 ± 3 (73)  |
| Composite       | 114 ± 2 (129) | 130 ± 2 (101) | 119 ± 2 (148) |
| Cell width, μm  |               |               |               |
| Septum          | 21.5 ± 0.4    | 23.1 ± 0.6    | 21.7 ± 0.5    |
| LV free wall    | 22.3 ± 0.6    | 22.7 ± 0.5    | 23.7 ± 0.5    |
| Composite       | 21.9 ± 0.3    | 22.9 ± 0.4    | 22.7 ± 0.3    |

Values are means ± SE. Numbers in parentheses are number of myocytes, without regard to number of cells contributed by each heart. MI, myocardial infarction; HIST, high-intensity sprint training; Sed, sedentary; Sham, sham operated; LV, left ventricle. For cell length, two-way ANOVA indicates significant group (Sham-Sed vs. MI-Sed vs. MI-HIST) effect ( $P < 0.0001$ ) but insignificant location (septum vs. LV free wall;  $P = 0.77$ ) and group × location interaction ( $P = 0.09$ ) effects. One-way ANOVA (composite; without regard to location) indicates significant ( $P < 0.0001$ ) differences among the mean cell lengths of 3 groups. A priori comparison of means indicates significant differences in cell lengths between Sham-Sed and MI-Sed ( $P < 0.0001$ ), between MI-Sed and MI-HIST ( $P < 0.0001$ ), and between Sham-Sed and MI-HIST ( $P < 0.04$ ) myocytes. For cell width, two-way ANOVA shows insignificant group ( $P = 0.16$ ), location ( $P = 0.06$ ), and group × location interaction ( $P = 0.07$ ) effects.

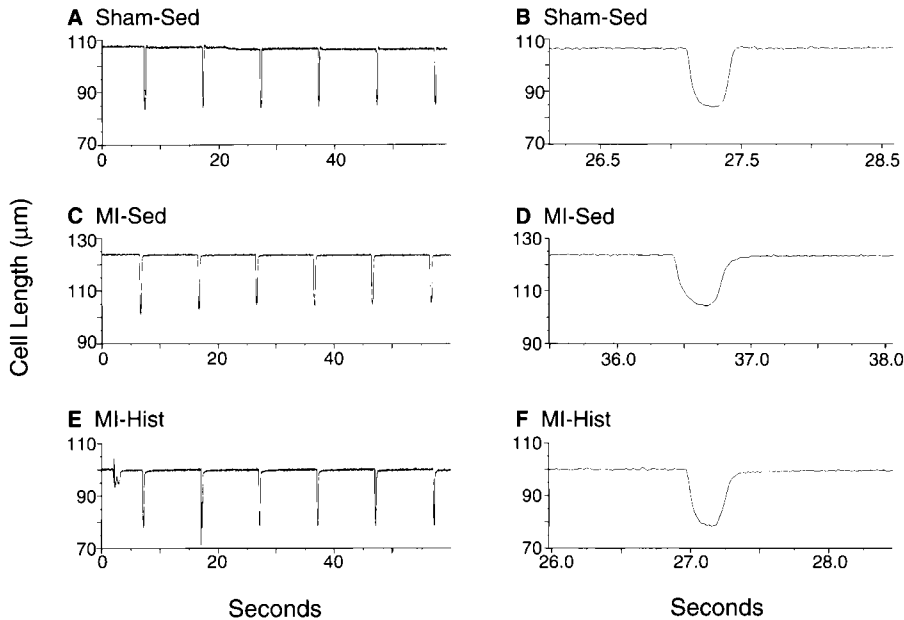


Fig. 1. High-intensity sprint training (HIST) improves contractile performance in myocytes from rats with induced myocardial infarction (MI). Isolated myocytes were paced (0.1 Hz) to contract at 37°C and extracellular  $Ca^{2+}$  concentration ( $[Ca^{2+}]_o$ ) of 5 mM. Shown are steady-state paced twitches of myocytes from sham-operated (Sham)-sedentary (Sed) (A), MI-Sed (C), and MI-HIST (E) rats and time-expanded views of steady-state twitches from which peak contraction amplitude, time required to reach half maximal contraction amplitude (time to half peak), shortening velocity ( $-dL/dt$ ), relengthening velocity ( $+dL/dt$ ), and half time of relaxation were measured for Sham-Sed (B), MI-Sed (D), and MI-HIST (F) myocytes. Note that HIST restored peak contraction amplitude in MI myocytes to that observed in Sham-Sed myocytes. Results are summarized in Table 2.

length), time required to reach one-half maximal contraction amplitude (time to  $\frac{1}{2}$  peak), maximal shortening ( $-dL/dt$ ) and relengthening ( $+dL/dt$ ) velocities, and half time of relaxation ( $t_{1/2}$ ) can be calculated. To maximize the differences in contractile behavior between Sham-Sed and MI-Sed myocytes, experimental conditions were chosen to favor either  $Ca^{2+}$  influx over efflux pathways (5.0 mM  $[Ca^{2+}]_o$ , 0.1 Hz) or vice versa (0.6 mM  $[Ca^{2+}]_o$ , 2 Hz) (22, 33). Data are summarized in Table 2. Across the three groups, there were no differ-

ences in any of the measured contraction parameters between myocytes isolated from the septum and those isolated from LV free wall (three-way ANOVA demonstrates lack of location or group  $\times$  location effects). Therefore, it is reasonable to simplify our data analysis by removing myocyte origin (septum vs. LV free wall) from further consideration.

At 0.6 mM  $[Ca^{2+}]_o$  and 2 Hz, MI-Sed myocytes shortened 21.2% more than Sham-Sed myocytes (Fig. 2A). At 5.0 mM  $[Ca^{2+}]_o$  and 0.1 Hz, MI-Sed myocytes short-

Table 2. Summary of myocyte shortening dynamics data

| Location   | $[Ca^{2+}]_o$ | Sham-Sed              | MI-Sed                | MI-HIST               |
|--|---------------|-----------------------|-----------------------|-----------------------|
| Maximal contraction amplitude, % resting cell length |               |                       |                       |                       |
| Septum   | 0.6           | 5.33 $\pm$ 0.35 (31)  | 5.96 $\pm$ 0.44 (31)  | 6.19 $\pm$ 0.45 (33)  |
|  | 5.0           | 19.53 $\pm$ 0.50 (36) | 13.86 $\pm$ 0.85 (18) | 18.55 $\pm$ 0.57 (42) |
| LV free wall   | 0.6           | 5.20 $\pm$ 0.36 (30)  | 6.83 $\pm$ 0.43 (33)  | 6.67 $\pm$ 0.54 (28)  |
|  | 5.0           | 19.86 $\pm$ 0.53 (32) | 13.95 $\pm$ 0.89 (19) | 19.73 $\pm$ 0.58 (45) |
| Time to $\frac{1}{2}$ peak, ms                       |               |                       |                       |                       |
| Septum   | 0.6           | 47.4 $\pm$ 2.1        | 48.7 $\pm$ 1.3        | 45.1 $\pm$ 1.6        |
|  | 5.0           | 64.6 $\pm$ 2.8        | 82.7 $\pm$ 4.8        | 60.0 $\pm$ 1.8        |
| LV free wall   | 0.6           | 49.5 $\pm$ 1.8        | 51.4 $\pm$ 2.0        | 42.4 $\pm$ 1.1        |
|  | 5.0           | 60.2 $\pm$ 1.1        | 68.3 $\pm$ 2.4        | 62.3 $\pm$ 2.6        |
| Maximal $-dL/dt$ , cell length/s                     |               |                       |                       |                       |
| Septum   | 0.6           | 0.81 $\pm$ 0.05       | 0.91 $\pm$ 0.07       | 0.93 $\pm$ 0.07       |
|  | 5.0           | 2.09 $\pm$ 0.08       | 1.30 $\pm$ 0.08       | 1.93 $\pm$ 0.08       |
| LV free wall   | 0.6           | 0.79 $\pm$ 0.06       | 1.01 $\pm$ 0.08       | 1.10 $\pm$ 0.09       |
|  | 5.0           | 2.10 $\pm$ 0.07       | 1.31 $\pm$ 0.10       | 2.00 $\pm$ 0.07       |
| Maximal $+dL/dt$ , cell length/s                     |               |                       |                       |                       |
| Septum   | 0.6           | 0.72 $\pm$ 0.06       | 0.86 $\pm$ 0.07       | 0.88 $\pm$ 0.08       |
|  | 5.0           | 1.72 $\pm$ 0.08       | 0.87 $\pm$ 0.11       | 1.31 $\pm$ 0.07       |
| LV free wall   | 0.6           | 0.73 $\pm$ 0.06       | 0.88 $\pm$ 0.09       | 0.97 $\pm$ 0.09       |
|  | 5.0           | 1.72 $\pm$ 0.08       | 0.91 $\pm$ 0.10       | 1.43 $\pm$ 0.06       |
| Half time of relaxation, ms                          |               |                       |                       |                       |
| Septum   | 0.6           | 64.0 $\pm$ 4.2        | 59.2 $\pm$ 3.5        | 67.8 $\pm$ 4.4        |
|  | 5.0           | 121.6 $\pm$ 4.5       | 146.6 $\pm$ 13.0      | 130.9 $\pm$ 4.5       |
| LV free wall   | 0.6           | 61.5 $\pm$ 3.1        | 69.5 $\pm$ 4.6        | 60.3 $\pm$ 3.6        |
|  | 5.0           | 116.6 $\pm$ 3.9       | 149.1 $\pm$ 12.3      | 129.0 $\pm$ 4.2       |

Values are means  $\pm$  SE. Numbers in parentheses are numbers of myocytes, without regard to no. of cells contributed by each heart.  $[Ca^{2+}]_o$ , extracellular  $Ca^{2+}$  concentration; time to  $\frac{1}{2}$  peak, time required to reach one-half maximal contraction amplitude;  $-dL/dt$ , shortening velocity;  $+dL/dt$ , lengthening velocity.

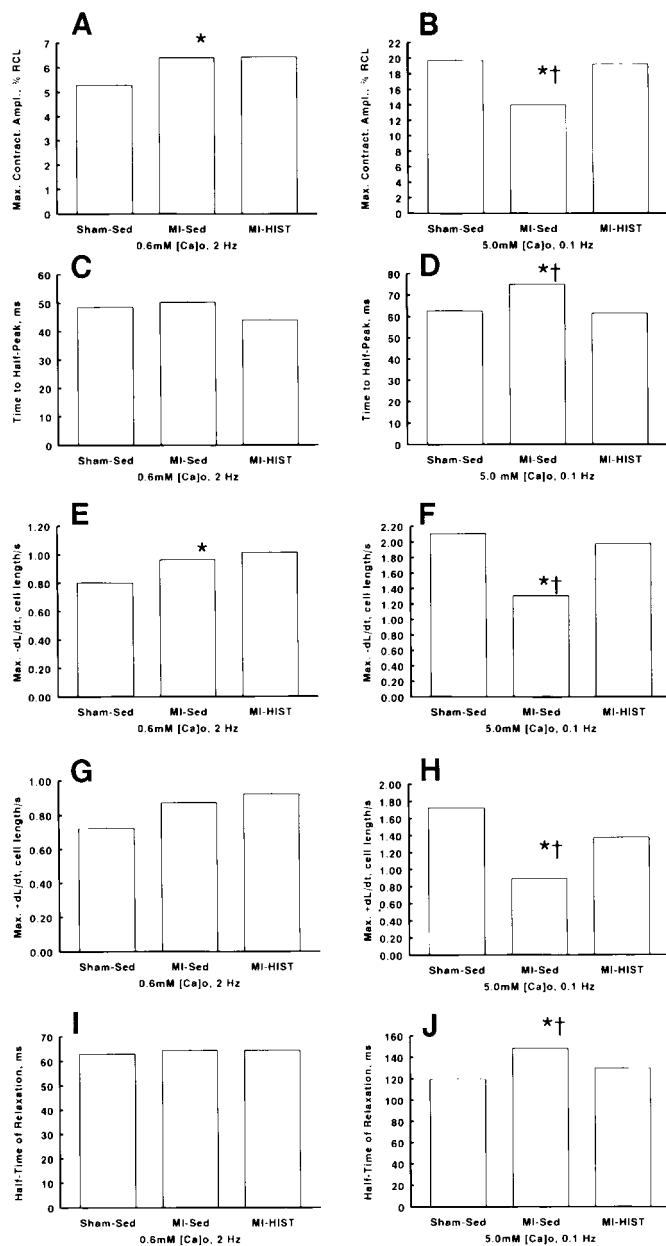


Fig. 2. Contraction parameters in Sham-Sed, MI-Sed, and MI-HIST myocytes. Maximal contraction amplitude (max contract ampl), time to  $\frac{1}{2}$  peak, maximal  $-dL/dt$ , maximal  $+dL/dt$ , and half time of relaxation are measured at 0.6 mM  $[Ca^{2+}]_o$  and 2 Hz (A, C, E, G, and I) and at 5.0 mM  $[Ca^{2+}]_o$  and 0.1 Hz (B, D, F, H, and J). Data for all myocytes are presented, without regard to location (septal vs. left ventricular free wall) because there was no location difference in any of the contraction parameters among the 3 groups by 3-way ANOVA. After significant differences were detected by 2-way ANOVA, post hoc analysis was performed to compare Sham-Sed vs. MI-Sed, MI-Sed vs. MI-HIST, and Sham-Sed vs. MI-HIST myocytes. %RCL, percent resting cell length. \* $P < 0.0002$ , Sham-Sed vs. MI-Sed; † $P < 0.03$ , MI-Sed vs. MI-HIST.

ened 29.3% less than Sham-Sed myocytes (Fig. 2B). These conclusions are supported by a significant group  $\times [Ca^{2+}]_o$  interaction effect ( $P < 0.0001$ ), indicating that the magnitude and/or direction of the effect of  $[Ca^{2+}]_o$  on cell shortening was different across experimental groups (Sham-Sed vs. MI-Sed). These ob-

servations are in agreement with our previously published results (33). HIST was effective in restoring normal myocyte contraction amplitude at high  $[Ca^{2+}]_o$  (5.0 mM) (Fig. 2B) but not at low  $[Ca^{2+}]_o$  (0.6 mM) conditions (Fig. 2A). Indeed, post hoc analysis between Sham-Sed and MI-HIST myocytes demonstrated insignificant group effects ( $P = 0.41$ ) but highly significant  $[Ca^{2+}]_o$  ( $P < 0.0001$ ) and group  $\times [Ca^{2+}]_o$  ( $P = 0.02$ ) interaction effects, indicating that the beneficial effects of HIST on contraction amplitude were manifest only at high- $[Ca^{2+}]_o$  and low-pacing-frequency conditions.

Significant differences existed among the three groups with respect to maximal  $-dL/dt$  (Fig. 2, E and F;  $P < 0.0001$ ) and  $+dL/dt$  (Fig. 2, G and H;  $P < 0.0001$ ). Post hoc analyses indicated significant group differences in maximal  $-dL/dt$  ( $P < 0.0001$ ) and  $+dL/dt$  ( $P < 0.0001$ ) between Sham-Sed and MI-Sed and between MI-Sed and MI-HIST myocytes ( $P < 0.0001$  for both  $-dL/dt$  and  $+dL/dt$ ) but not between Sham-Sed and MI-HIST myocytes ( $P = 0.45$  for  $-dL/dt$ ;  $P = 0.14$  for  $+dL/dt$ ). The differences in  $-dL/dt$  and  $+dL/dt$  could not be explained by the differences in contraction amplitudes observed in the three groups. In other words, between-group differences were still significant when shortening and relengthening rates were normalized for the amplitude of the contraction (normalized  $-dL/dt$ , group main effects,  $P = 0.01$ ; normalized  $+dL/dt$ , group main effects,  $P = 0.01$ ). Similar to observations on contraction amplitudes, the efficacy of HIST on restoring  $-dL/dt$  and  $+dL/dt$  to normal was  $[Ca^{2+}]_o$  dependent, as indicated by insignificant group but highly significant group  $\times [Ca^{2+}]_o$  interaction effects (Sham-Sed vs. MI-HIST,  $P = 0.0002$  for  $-dL/dt$ ,  $P < 0.0001$  for  $+dL/dt$ ).

Time to  $\frac{1}{2}$  peak was significantly ( $P < 0.0001$ ) longer in MI-Sed myocytes compared with Sham-Sed myocytes (Fig. 2, C and D), and the differences were larger at higher  $[Ca^{2+}]_o$  (group  $\times [Ca^{2+}]_o$  interaction effect,  $P = 0.002$ ). HIST was effective in reducing time to  $\frac{1}{2}$  peak in MI myocytes (Fig. 2, C and D; MI-Sed vs. MI-HIST,  $P < 0.0001$ ).

Compared with Sham-Sed myocytes, MI-Sed myocytes had longer  $t_{1/2}$  (Fig. 2, I and J;  $P = 0.0002$ ); the differences were magnified under high  $[Ca^{2+}]_o$  conditions (group  $\times [Ca^{2+}]_o$  interaction effects,  $P = 0.001$ ). HIST was able to restore  $t_{1/2}$  in MI myocytes close to that measured in Sham-Sed myocytes (Fig. 2, I and J; MI-Sed vs. MI-HIST,  $P = 0.03$ ).

## DISCUSSION

There are major differences among the various MI models utilized by various investigators to study excitation-contraction abnormalities in surviving myocytes, depending on the animal species, infarct sizes, experimental conditions, and time of recovery from acute MI (2, 3, 6, 15–18, 20, 25, 30–36). This wide spectrum of changes was also observed in failing human hearts, in which at least two different phenotypes existed at both ends of the spectrum: systolic dysfunction alone vs. systolic and diastolic dysfunction (14). To

facilitate discussion of the mechanisms by which HIST may improve MI myocyte contractile performance, it would be fruitful to summarize known excitation-contraction abnormalities in our rat MI model. Compared with Sham myocytes, in MI myocytes' action potential duration was prolonged (37),  $I_{Ca}$  characteristics were unchanged (32),  $I_{Na/Ca}$  was depressed (36) although there were no detectable changes in  $Na^+/Ca^{2+}$  exchange protein levels (35), SR  $Ca^{2+}$  uptake was lower concomitant with lower levels of sarco(endo)plasmic reticulum  $Ca^{2+}$ -ATPase (SERCA2; Ref. 34), SR  $Ca^{2+}$  content was reduced (36), myosin heavy chain isoenzyme shifted from  $V_1$  to  $V_3$  pattern (24, 35), and contractile performance was impaired (6, 33). It should be noted that many of our findings in the rat MI model have been independently corroborated by the work of Afzal and Dhalla (2). It should also be pointed out that the phenotypic manifestations (decreased SERCA2 but no change in  $Na^+/Ca^{2+}$  exchange protein levels) of our rat MI model are consistent with those observed in group III failing human hearts as defined by Hasenfuss et al. (14), in which both systolic and diastolic dysfunction were present (in our case, lower contraction amplitude and longer  $t_{1/2}$ , Fig. 2, B and J).

Compared with Sham-Sed myocytes, at high  $[Ca^{2+}]_o$  and low pacing frequency, MI-Sed myocytes contracted ~30% less. In addition, there was significant prolongation of contraction, both in terms of time to  $\frac{1}{2}$  peak and half time of relaxation. In 6-wk MI rats, the time to peak tension measured in the noninfarcted LV posterior papillary muscle was prolonged compared with that in sham-operated rats (19). The prolonged time to  $\frac{1}{2}$  peak might be due to continued transsarcolemmal  $Ca^{2+}$  influx (via  $I_{Ca}$  and reverse  $I_{Na/Ca}$ ) because of prolonged action potential duration, prolonged SR  $Ca^{2+}$  release, depressed SR  $Ca^{2+}$  uptake, or reduced  $Ca^{2+}$  extrusion by forward  $Na^+/Ca^{2+}$  exchange. The prolonged action potential duration in MI-Sed myocytes (18, 37) might be a mechanism to allow more  $Ca^{2+}$  entry via reverse  $Na^+/Ca^{2+}$  exchange during the plateau phase of the action potential. This interpretation received indirect support in that, when pacing frequency was increased to 2 Hz with proportional shortening of action potential duration, the time to  $\frac{1}{2}$  peak was no longer different between Sham-Sed and MI-Sed myocytes. This simplistic interpretation must be viewed in the context that during a cardiac action potential, there are complicated interactions between  $I_{Ca}$  and  $I_{Na/Ca}$  and that it is extremely difficult to fully characterize these two (and other) currents during an action potential. A prolonged  $t_{1/2}$  may be explained by reduced SR  $Ca^{2+}$  uptake, decreased  $Ca^{2+}$  extrusion by forward  $Na^+/Ca^{2+}$  exchange, and lower myosin ATPase activity in MI-Sed myocytes. It is interesting to note the important role  $Na^+/Ca^{2+}$  exchange played in both contraction and relaxation in diseased myocytes, as has been recently demonstrated in failing human myocytes (9, 12, 14).

Our study is the first to demonstrate that a program of exercise training instituted shortly after recovery from acute MI was effective in improving cardiac con-

tractile performance at the level of a single myocyte. Concerns about the clinical appropriateness and safety of high-intensity exercise training post-MI have been previously addressed, both in humans (1, 10) and animal models (23, 30, 35). Focusing on the rat MI model, we have previously demonstrated that 6–8 wk of HIST effected significant increases in cardiac output and maximal stroke volume in post-MI hearts (23). Although systemic factors (central cardiorespiratory and peripheral vascular adaptations, and so forth) likely contributed to HIST-induced improvements in cardiac performance in post-MI rats, cellular adaptations to HIST may be equally important in improved myocardial function. Indeed, HIST has been shown to exert beneficial effects on multiple cellular events involved in excitation-contraction coupling in post-MI myocytes, including but not limited to restitution of normal isoenzyme distribution of contractile proteins, restoring normal action potential duration, enhancing  $I_{Na/Ca}$ , normalizing  $[Ca^{2+}]_i$  transients during a twitch, improving SR  $Ca^{2+}$  uptake, and increasing SR  $Ca^{2+}$  contents (30, 35, 37).

In our present study, we did not detect significant differences in cell lengths, cell widths, and contraction parameters between myocytes isolated from septum and those from LV free wall in the three groups studied (lack of location effect; see Tables 1 and 2). In a previous study from our laboratory, whole cell capacitance measurements also failed to support myocyte origin (septum vs. LV free wall) as an important determinant in capacitance values in MI myocytes (30). Our findings are in agreement with those of Lefroy et al. (15) but not with those of Olivetti et al. (25). In any event, lack of location and group  $\times$  location interaction effects in all contraction parameters examined makes it reasonable to discuss our present data without reference to location differences.

The observation that MI-Sed myocytes were ~14% longer than Sham-Sed myocytes but cell widths were similar between the two groups (Table 1) was in agreement with our previous report (6). Other investigators also reported that increases in cell lengths were larger than increases in cell widths in post-MI myocytes (3, 18, 20, 25), a characteristic consistent with volume-overload hypertrophy (4). The first major finding is that HIST was effective in attenuating cellular hypertrophy in MI-Sed myocytes, primarily by decreasing cell lengths with no effects on cell widths (Table 1). It is interesting to speculate that, by directly modulating cell hypertrophy in postinfarction myocytes, HIST may minimize ventricular dilation post-MI. Indeed, 6 wk of graduated swim training attenuated LV dilation by ~30% in post-MI rat hearts (26). It is also interesting to note the "dual" effects of exercise training on cardiac myocyte size: physiologic hypertrophy in normal myocytes (21, 22) and regression of pathologic hypertrophy in post-MI myocytes (30, 35; Table 1).

The second major finding is that HIST was effective in restoring normal contraction amplitude in post-MI myocytes stimulated at 5.0 mM  $[Ca^{2+}]_o$  and 0.1 Hz. Under these experimental conditions, HIST has been shown to

increase  $I_{Na/Ca}$ , systolic  $[Ca^{2+}]_i$ , SR  $Ca^{2+}$  uptake, and SR  $Ca^{2+}$  content in post-MI myocytes (30, 35). Positive influences by HIST on all these cellular mechanisms involved in excitation-contraction coupling may enhance contractile performance in post-MI myocytes. On the other hand, at 0.6 mM  $[Ca^{2+}]_o$  and 2 Hz, HIST did not attenuate the “supranormal” contraction amplitude observed in MI-Sed myocytes (Fig. 2A). This may be due to the fact that the major  $Ca^{2+}$  efflux pathway,  $I_{Na/Ca}$ , was not restored to normal levels by HIST (35); that  $Na^+/Ca^{2+}$  exchange assumed a more important role in contraction and relaxation in diseased myocytes (12, 14); that HIST normalized the isoenzyme distribution patterns (and, by inference, contractile efficiency) of contractile proteins (23, 35); or that exercise training increased the  $Ca^{2+}$  sensitivity of myofilaments (22), which could be subnormal in MI myocytes (16), although the last point is controversial (3).

The time to reach one-half maximal contraction amplitude was prolonged in MI-Sed myocytes (Fig. 2D), similar to the findings in surviving myocytes isolated from healing canine infarcted hearts (14) and 6-wk MI rats (19). HIST was effective in reducing time-to- $\frac{1}{2}$  peak in MI myocytes, probably by shortening action potential duration (37) and enhancing SR  $Ca^{2+}$  uptake (30) and sarcolemmal  $Ca^{2+}$  extrusion by  $Na^+/Ca^{2+}$  exchange (35). In this light, it should be pointed out that improvement in  $I_{Ca}$  by HIST in MI myocytes is an unlikely mechanism for reducing time to  $\frac{1}{2}$  peak shortening. Our laboratory has previously shown that the intrinsic properties of  $I_{Ca}$  were not different between Sham and MI myocytes (32) and that endurance training (albeit not HIST) did not modify  $I_{Ca}$  characteristics in running rats (21).

Another important finding of the present study is that HIST shortened  $t_{1/2}$ , which was prolonged in MI-Sed myocytes (Fig. 2J). Longer  $t_{1/2}$  in MI-Sed myocytes, compared with Sham-Sed myocytes, was expected in view of previous findings that SR  $Ca^{2+}$  uptake (2, 34), SR CaATPase expression (34), and  $Na^+/Ca^{2+}$  exchange activity (36) were reduced in MI rat myocytes. Improvement in SR  $Ca^{2+}$  uptake (30) and forward  $Na^+/Ca^{2+}$  exchange (35) in MI-Sed myocytes by HIST may contribute to the reduction of  $t_{1/2}$ . This simple interpretation must be tempered by the fact that, in rabbit, rat (5), and canine (17) cardiac myocytes, relaxation was complete well before the end of the  $[Ca^{2+}]_i$  transient. For example, in rat myocytes stimulated at 1.0 mM  $[Ca^{2+}]_o$  and 22°C,  $t_{1/2}$  from a twitch was  $0.08 \pm 0.01$  s, whereas the time constant of  $[Ca^{2+}]_i$  transient decline was  $0.194 \pm 0.010$  s (5). Under our experimental conditions of 5.0 mM  $[Ca^{2+}]_o$  and 37°C,  $t_{1/2}$  from a twitch was  $120 \pm 3$  ms (Fig. 2J) but half time of  $[Ca^{2+}]_i$  decline was  $197 \pm 5$  ms (30). The fact that myocyte relaxation was complete well ahead of  $[Ca^{2+}]_i$  transient suggests that mechanisms in addition to enhanced SR  $Ca^{2+}$  uptake and forward  $Na^+/Ca^{2+}$  exchange contribute to improved myocyte mechanical relaxation.

Finally, there are a number of important limitations to this study. The first is that, compared with Sham myocytes, surviving myocytes isolated from our rat MI model did not exhibit significant contraction amplitude differences when stimulated at “physiological”  $[Ca^{2+}]_o$ ,

of 1.1–1.9 mM and at 0.1–5 Hz (6, 33). Absence of significant differences in twitch amplitudes between Sham and rat MI myocytes was also reported by Anand et al. (3). However, contraction abnormalities in our rat MI myocytes could clearly be demonstrated under high  $[Ca^{2+}]_o$  and low pacing frequencies and also at low  $[Ca^{2+}]_o$  and high pacing frequencies (33; Fig. 2). We interpreted the complex pattern of contraction abnormalities to be consistent with the notion that  $Ca^{2+}$  influx and efflux pathways were reduced in MI myocytes. In view of this, together with our laboratory’s previous observation that HIST improved  $Na^+/Ca^{2+}$  exchange activity (which affects both  $Ca^{2+}$  influx and efflux) in MI myocytes (35), in the present study we purposefully examined the potential beneficial effects of HIST on MI myocyte contraction under conditions that favored  $Ca^{2+}$  influx over efflux (high  $[Ca^{2+}]_o$  and low pacing frequency) and vice versa (low  $[Ca^{2+}]_o$  and high pacing frequency). The second limitation relates to the fact that we did not examine the effects of HIST on Sham myocytes. Although it is of considerable interest to delineate potential changes in excitation-contraction coupling in normal myocytes by HIST, previous (30, 35) and present focus by our laboratory continues to be the elucidation of cellular mechanisms by which HIST improved global myocardial contractile function in post-MI rats (23). The third limitation concerns the fact that in the study of contraction abnormalities of single myocytes isolated from MI hearts, it is difficult to ascertain for each individual myocyte how close to or far away from the infarcted area it was. Theoretically, surviving myocytes close to the infarct may be subjected to greater hemodynamic stress during recovery and may thus exhibit different cellular adaptation from those distant from the infarct. As a first approach, we separately examined myocytes isolated from the septum (spared and distant from infarct) and the LV free wall (close to infarct). There were no differences in myocyte dimensions and contraction parameters between myocytes isolated from septum and those from LV free wall in the three groups studied (Tables 1 and 2). The fourth limitation is that there were no direct measurements of  $[Ca^{2+}]_i$  transients in myocytes from the three experimental groups. In a previous study (30), our laboratory has shown that, at 5.0 mM  $[Ca^{2+}]_o$ , HIST was effective in restoring the depressed systolic  $[Ca^{2+}]_i$  and lowering the elevated diastolic  $[Ca^{2+}]_i$  of MI-Sed myocytes toward those observed in Sham-Sed myocytes. Thus the observed improvements in contraction amplitudes by HIST in the present study may be related to its beneficial effects on  $Ca^{2+}$  homeostasis in MI myocytes.

In summary, we have demonstrated that HIST attenuated cellular hypertrophy post-MI, primarily by reduction in cell lengths with little effect on cell widths. In our rat MI model, which exhibited both systolic and diastolic dysfunction, HIST was effective in improving many of contraction parameters measured in MI myocytes in the unloaded state. We hypothesize that enhanced  $Na^+/Ca^{2+}$  exchange activity, reduced action potential duration, and improved SR  $Ca^{2+}$  uptake may

be some of the more important mechanisms responsible for improved contractile performance by HIST in post-MI myocytes.

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