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Altered single cell force-velocity and power properties in exercise-trained rat myocardium

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Diffee, Gary M., and Eunhee Chung. Altered single cell force-velocity and power properties in exercise-trained rat myocardium. *J Appl Physiol* 94: 1941–1948, 2003. First published January 10, 2003; 10.1152/japplphysiol.00889.2002.—Myocardial function is enhanced by endurance exercise training, but the cellular mechanisms underlying this improved function remain unclear. The ability of the myocardium to perform external work is a critical aspect of ventricular function, but previous studies of myocardial adaptation to exercise training have been limited to measurements of isometric tension or unloaded shortening velocity, conditions in which work output is zero. We measured force-velocity properties in single permeabilized myocyte preparations to determine the effect of exercise training on loaded shortening and power output. Female Sprague-Dawley rats were divided into sedentary control (C) and exercise trained (T) groups. T rats underwent 11 wk of progressive treadmill exercise. Myocytes were isolated from T and C hearts, chemically skinned, and attached to a force transducer. Shortening velocity was determined during loaded contractions at 15°C by using a force-clamp technique. Power output was calculated by multiplying force times velocity values. We found that unloaded shortening velocity was not significantly different in T vs. C myocytes (T = 1.43 muscle lengths/s, n = 46 myocytes; C = 1.12 muscle lengths/s, n = 43 myocytes). Training increased the velocity of loaded shortening and increased peak power output (peak power = 0.16 P/P_o × muscle length/s for T myocytes; peak power = 0.10 P/P_o × muscle length/s for C myocytes, where P/P_o is relative tension). We found no effect of training on myosin heavy chain isoform content. These results suggest that training alters power output properties of single cardiac myocytes and that this adaptation may improve the work capacity of the myocardium.

myocardial function; treadmill exercise

CHRONIC ENDURANCE EXERCISE TRAINING has been shown to increase the functional capacity of the heart, as evidenced by greater maximal cardiac output and greater maximal and submaximal stroke volume (2, 16, 30). This increase in stroke volume is thought to be due, in part, to training-induced alterations of the intrinsic contractile function of the myocardium (4, 11, 16). However, the nature and potential mechanisms of these alterations in contractile function remain incompletely characterized. A number of studies examining the effect of training on the contractile performance of myocardial muscle preparations or single cardiac cells have indicated that training increases the tension-generating capability of the myocardium (6, 8, 24, 35), but these studies have only examined tension production under isometric conditions in which the muscle does not shorten. A small number of studies have measured unloaded shortening properties of single myocytes. Some studies have found that exercise training increases the extent of shortening under some conditions but not the rate of shortening (20, 36), whereas others have found no effect on either the rate or extent of shortening (17, 24, 26). A larger number of studies have measured the effect of training on myosin ATPase activity, which is the biochemical correlate of maximum shortening velocity (1). These studies have given conflicting results, with a number of studies reporting a training-induced increase in ATPase activity as well as a number showing no effect of training on ATPase activity (reviewed in Ref. 20). In any case, these previous studies have not addressed the effect of training on the ability of the myocardium to shorten under a load.

The ability of the heart to pump blood through the circulation depends on the capacity of the ventricle to perform external work. Thus, to eject blood from the heart, the myocardium must shorten under load. The velocity at which muscle shortens is inversely proportional to the force that the muscle must produce, with the relationship between force and shortening velocity (i.e., the force-velocity curve) being generally described by a rectangular hyperbola (15). Power output (work per unit time) is the product of force and velocity. Thus myocardial power output occurs only during loaded shortening, and power is zero at both zero force (maximal unloaded shortening) and at zero velocity (isometric tension). As mentioned above, most studies of effects of exercise training on myocardial contractile properties have focused on isometric tension or maximal shortening velocity where power output is zero. Thus there is very little that is known about the effect of exercise training on force-velocity or power output properties in the myocardium. One earlier study (19) examined force during loaded shortening in a multicellular myocardial preparation and found that exercise training changed the shape of the force-velocity curve

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and increased maximal shortening velocity and loaded shortening velocity, but no assessment was made regarding the effect of these changes on work or power output. In addition, multicellular preparations such as papillary muscles often contain extracellular and other viscoelastic elements that confound isotonic shortening measurements. The aim of the present study was to determine the effect of exercise training on force-velocity properties and power output in single permeabilized cardiac myocytes. Removal of membranes and extracellular elements allows one to focus directly on training-induced adaptations of the contractile element.

METHODS

Exercise training protocol. Female Sprague-Dawley rats were randomly divided into a control group ($n = 7$) and a training group ($n = 7$). The animals were housed in individual cages on a 12:12-h light-dark cycle and had access to food and water ad libitum. Training consisted of an 11-wk treadmill training protocol that had previously been shown to increase maximal oxygen uptake and increase cardiac performance at the whole heart (11), myocardial (35), and single cell level (6, 8). Rats were trained on a rodent treadmill starting at 15 min/day at a speed of 10 m/min and a 10% grade. The intensity and duration of the training sessions were progressively increased until, at *week 6*, the animals were running at 26 m/min and up a 20% grade for 1 h/day. This intensity and duration were then maintained though the final 5 wk. This protocol received approval from the University of Wisconsin-Madison Animal Use and Care Committee.

Cardiac myocyte preparation. For contractile measurements, single myocyte-sized preparations were obtained by mechanical disruption of ventricular tissue as described previously (8). Animals were anesthetized by inhalation of methoxyflurane, and the hearts were quickly excised and weighed. The heart was placed in ice-cold Ca^{2+}-free relaxing solution; trimmed of atria, connective tissue, and vascular tissue; and cut into three sections. These sections were quick frozen in liquid nitrogen and stored at −80°C until used to prepare myocytes for contractile measurements. On the day of an experiment, one heart section was placed into ~30 ml of ice-cold relaxing solution, minced with scissors, and further disrupted in a Waring blender. The resulting suspension of cells and cell fragments was centrifuged, and the pellet was then resuspended in cold relaxing solution plus 1% Triton X-100 for 7 min. The resulting skinned myocytes were resuspended in 8–10 ml of relaxing solution and kept on ice throughout the days of the experiments. All contractile experiments were performed within 48 h of cell preparation. Cells were discarded after the second day. Crucial to the success of this experimental approach was the ability to obtain viable myocytes from frozen tissue. Our laboratory has previously reported that there was no difference between the isometric tension properties of myocytes isolated from trained vs. control tissue (8). For this study, we determined that there were also no differences in shortening properties between cells prepared from frozen hearts and cells prepared from fresh hearts, nor were there differences in contractile properties of first-day vs. second-day cells (data not shown).

Experimental apparatus. The experimental apparatus has been described previously (8). Skinned cardiac myocyte preparations were attached between a capacitance-gauge transducer (model 403, Aurora Scientific) and a direct-current torque motor (model 308, Aurora Scientific) by placing the ends of the preparation into stainless steel troughs. The ends were then secured to the troughs by overlaying an ~0.5-mm length of 4-0 monofilament suture over each end and then tying the suture to the trough by using a loop of 10-0 monofilament suture.

The experimental preparation was viewed by using an inverted microscope (Olympus IX50) with a ×20 objective and fitted with a ×15 black and white photoeyepiece (Sony CCD-IRIS). A video image of the myocyte was displayed on a monitor, and sarcomere length was measured by using a micrometer against this image (Fig. 1). All experiments were conducted with sarcomere length set to 2.3 μm. Length and force changes during contractile measurements were driven by voltage commands from a personal computer via a 16-bit digital-to-analog converter. Force and length signals were digitized at 1 kHz by using a 16-bit analog-to-digital converter and were displayed and stored on a personal computer using custom software in LABVIEW for Windows (National Instruments). The experimental chamber contained three wells. The myocyte was moved from well to well to change bathing solutions. The experimental apparatus was cooled to 15°C by using a Peltier device (Cambion, Cambridge, MA) and a circulating water bath.

Solutions. Relaxing and activating solutions for skinned myocyte preparations contained 7 mM EGTA, 1 mM free Mg^{2+}, 20 mM imidazole, 4 mM ATP, 14.5 mM creatine phosphate, pH 7.0 (at 15°C), free Ca^{2+} concentration of either 10^{-9} M (relaxing solution) or $10^{-4.5}$ M (maximally activating

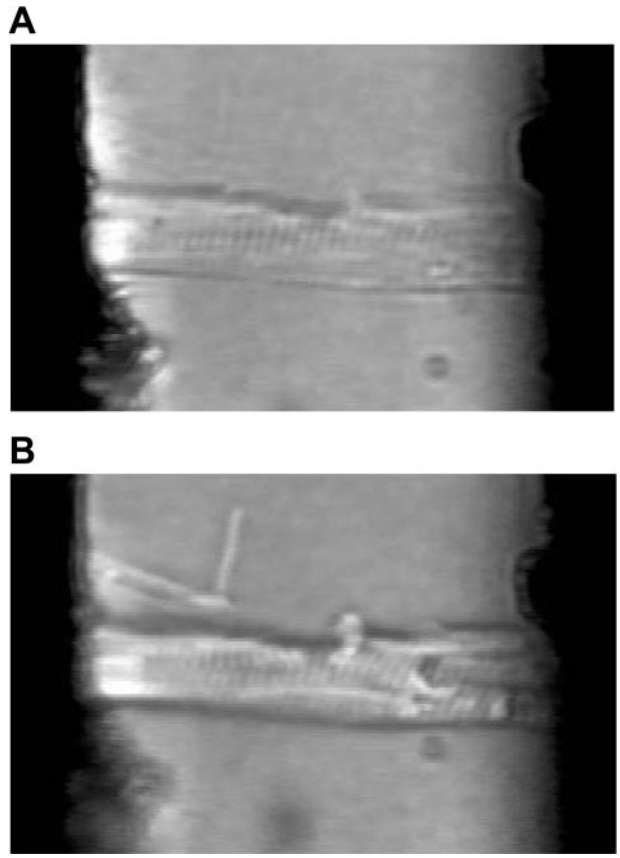

Fig. 1. Photomicrograph of a representative skinned myocyte mounted in the experimental apparatus. *A*: myocyte bathed in relaxing solution (pCa 9.0). *B*: same myocyte generating isometric force in maximally activating (pCa 4.5) solution.

solution), and sufficient KCl to adjust ionic strength to 180 mM. The final concentrations of each metal, ligand, and metal-ligand complex were determined from the computer program of Fabiato (10).

Force-velocity measurements. Loaded shortening measurements were made by using a modification of the technique described in Ref. 18. The servomotor was equipped with a force-link circuit (ASI2100A, Aurora Scientific) that utilized the signal from the force transducer to control the load on the myocyte. The myocyte was transferred into activating solution (pCa 4.5), and steady tension was allowed to develop. The computer then switched the motor from length control mode to force control mode by applying a 5-V logic pulse. The myocyte was rapidly stepped to a specified force less than maximal, and force was maintained at this level for 100–300 ms while changes in myocyte length were monitored. After this force clamp, the myocyte was slackened such that force fell to zero to allow measurement of the relative force during the isotonic shortening period. Several (7–10) force clamps at a variety of loads were done for each myocyte. Maximal force was monitored for each force clamp to assess any decline in force-producing capability of the myocyte. If maximal force declined by >15% during the experimental protocol, that cell was discarded and the data were not used. We found that force was maintained to a greater extent if the myocyte remained in activating solution for the entire sequence of force clamp measurements, similar to results seen by others (18).

Data analysis. Length data were expressed in terms of muscle (cell) length to correct for differences in the lengths of attached cells. Changes in length obtained during isotonic shortening were analyzed by regression analysis to determine the slope of the length change per unit time (shortening velocity). Force and velocity data were fitted to the Hill equation (15)

$$(P + a)(V + b) = (P_o + a)b$$

where P is force during shortening at velocity (V), P_o is the peak isometric force, and a and b are constants with the dimensions of force and velocity, respectively. Power load curves were obtained by multiplying force times velocity at each load. The optimum force for mechanical power output (F_{opt}) was calculated by using the equation (37)

$$F_{opt} = (a^2 + a\,P_o)^{1/2} - a$$

Data were fit to equations by using commercial software (SigmaPlot, Jandel Scientific).

Analysis of myosin heavy chain isoform content. Myosin heavy chain (MHC) isoform content of ventricular homogenates was determined by using a modification of a previously described SDS-polyacrylamide gel electrophoresis technique using *N-N′*-diallyltartardiamide (DATD) as a cross-linker rather than bisacrylamide (3). The use of DATD in the resolving gel was based on the description of its use in stacking gels (12) as well as the observation that addition of this cross-linker to low-percentage resolving gels improves the separation of MHC isoforms (M. Greaser and C. Warren, personal communication).

Homogenates of ventricular tissue (4–10 μg of protein per lane) were heated (3 min at 100°C), combined with sample buffer (8 M urea, 2 M thiourea, 0.05 M Tris, pH 6.8, 75 mM dithiothreitol, 3% SDS, and 0.05% bromophenol blue), and loaded onto polyacrylamide gels. Stacking gels were composed of 3% acrylamide (acrylamide-to-DATD ratio = 5.6:1), 10% glycerol, 130 mM Tris, pH 6.8, and 0.1% SDS. Resolving gels were composed of 6% acrylamide (acrylamide-to-DATD ratio = 37.5:1), 10% glycerol, 0.37 M Tris, pH 8.8, and 0.1% SDS. Gels were run by using SE 200 Tall (10 × 12 cm) Mighty Small Mini-Vertical Units (Hoefer) with 0.75-cm-thick spacers and an EPS 301 power supply (Amersham Pharmacia Biotech). The upper running buffer consisted of 100 mM Tris (base), 150 mM glycine, and 0.1% SDS. The lower running buffer consisted of 50 mM Tris (base), 75 mM glycine, and 0.05% SDS. The gels were run at 16 mA per gel (constant current) for 4 h at 4°C. Gels were silver stained by using Bio-Rad Silver Stain Plus kit according to kit instructions. Stained gels were dried down and scanned into bitmap file format by using an Epson Perfection 1200 Photoscanner with its transparency adapter (backlit). Density of bands was quantified from the bitmap file by using Un-Scan-It gel quantification software (Silk Scientific, Orem, UT). Density of the bands corresponding to the two MHC isoforms is expressed as a percentage of the total of both bands.

Citrate synthase. Plantaris muscles were removed after excision of the heart, trimmed of connective tissue, quick frozen in liquid nitrogen, and stored at −80°C. The plantaris muscles were thawed and homogenized in potassium phosphate buffer (pH 7.4) and assayed for citrate synthase activity at 25°C as previously described (33).

Statistical analysis. The statistical treatment of physiological data obtained from single cells has varied widely in studies of adaptation. Some studies have presented mean data from all cells within a given treatment group (21, 26), which gives a measure of cell-to-cell variability. Others have treated data from all cells in a given animal as a single data point (6, 8) and presented mean data from each animal, which gives a measure of animal-to-animal variability. In this study, we present pooled data from all myocytes within a given group (trained vs. control), and comparisons between the data from trained and control groups were made by using a one-way ANOVA with post hoc analysis, with $P < 0.05$ used to indicate a significant difference. These data are presented in Table 2. Data in Fig. 3*A* were obtained by plotting mean velocity values at each force value measured for all of the trained and control myocytes used in the study, resulting in a composite force-velocity curve (Fig. 3*A*). Similarly, power output values were obtained in each myocyte at each load by multiplying force times velocity values, and these data were used to construct the power-load curve (Fig. 3*B*).

RESULTS

The treadmill-training program used in this study elicited typical training effects in the rats as shown in Table 1. There was no significant difference in body weight between trained and control rats either before or after the 11-wk treadmill-training program. However, training did elicit a 12% increase in absolute heart weight and an 8% increase in the heart weight-to-body weight ratio. In addition, the plantaris muscles taken from the trained animals showed a 42% higher citrate synthase activity compared with control plantaris muscle. These values were all significantly different in trained compared with control animals ($P < 0.05$).

Figure 2 shows force and length traces for four representative force clamp measurements (to 0.20, 0.4, 0.6, and 0.80 P_o) in the same myocyte. As described above, myocytes were allowed to develop steady tension in maximally activating solution (pCa 4.5), and then the motor was switched from length control to force control mode. Force was stepped down to a pre-

Table 1. *Effect of exercise training on rat heart and skeletal muscle characteristics*

	Control (n = 7)	Trained (n = 7)
Body wt (pre), g	200.1 ± 6.9	198.4 ± 8.6
Body wt (post), g	256.2 ± 14.0	265.9 ± 12.9
Heart wt, g	0.687 ± .026	0.771 ± .017*
Heart wt/body wt × 1,000	2.69 ± .064	2.90 ± .081*
Plantaris citrate synthase activity, μmol·min^{-1}·g wet wt^{-1}	15.07 ± 0.59	21.44 ± 1.68*

Values are means ± SD; n, no. of rats. Pre, before exercise training; post, after exercise training. *Significantly different from control ($P < 0.05$).

selected value, and length changes were monitored. The myocyte was then slackened so that force fell to zero. As shown in Fig. 2*A*, length traces during isotonic shortening were well fit with a straight line. Previous studies in single myocytes have indicated that isotonic shortening is linear under maximally activating conditions but may be curvilinear under submaximal activating conditions (18). Linearity of shortening traces may also be taken as evidence of the relatively low compliance of attachment of our myocyte preparations (5, 31). The slope of the line fit to the isotonic shortening trace was taken as the shortening velocity for that load. These velocity values are plotted against force values for a single myocyte in Fig. 2*B*. The line is the best fit to the Hill equation as described in METHODS.

Data from force-velocity experiments are presented in two ways. First, data for each cell were fitted to the Hill equation as described in METHODS. This analysis resulted in a value for maximal unloaded shortening velocity (V_{max}) as well as a value for the term a/P_o (a measure of the curvature of the force-velocity curve). Conversion of force-velocity values to power output resulted in values for peak absolute power output, peak normalized power output, and F_{opt}. All of these values were then summed for each cell from trained (n = 46) and control (n = 43) groups. These data are presented in Table 2 along with maximal tension data. We found no significant differences between the trained and control groups in myocyte maximal tension or V_{max} but did find a significant increase ($P < 0.05$) in the values for a/P_o, peak power output (both absolute and normalized to P_o), and F_{opt}.

The above analysis provides quantifiable data that can be summed between animals (Table 2). However, Fig. 3*A* shows an alternative way of presenting this data that may provide a better illustration of the effect of training on force-velocity properties and power output, and also gives some indication of cell-to-cell variability. The graph in Fig. 3*A* is a composite force-velocity curve showing means ± SD velocity data at each relative force value for all of the trained (n = 46) and control (n = 43) myocytes. Mean force and velocity data were then fit with the Hill equation (shown by solid and dashed lines), resulting in cumulative V_{max} and a/P_o or trained and control myocytes. With the use of this analysis, data from the control myocytes yielded

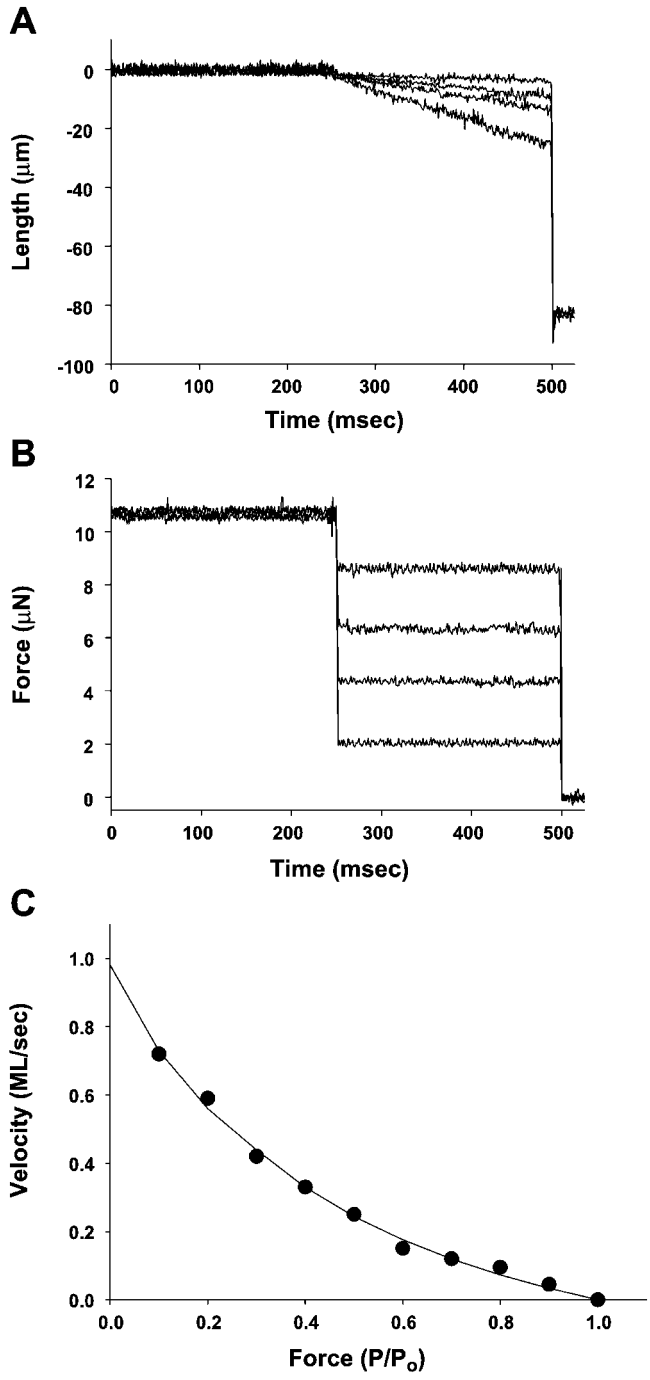

Fig. 2. Length (*A*) and force (*B*) traces from 4 force clamp experiments in a representative myocyte. After steady tension had developed in maximally activating solution (pCa 4.5), the servomotor was switched to force-control mode, and the force was stepped down to a preselected value [in this example, maximum isometric forces (P_o) of 0.8, 0.6, 0.4, and 0.2]. Length changes during isotonic shortening at each load were fit by using linear regression with the slope of the line taken as the velocity of shortening for that load. *C*: force-velocity curve in a single myocyte resulting from force-clamp experiments illustrated in *B*. A total of 9 force-clamp measurements were done for this myocyte. Data were fit to the Hill equation (line), and this resulted in an extrapolated maximal unloaded shortening velocity value (y-intercept) of 0.98 muscle lengths (ML)/s for this cell and an a/P_o value (measure of the curvature of the force-velocity relationship) of 0.39. P/P_o, relative tension.

Table 2. *Mechanical properties of myocytes isolated from trained and control animals*

	Maximal Force, kN/m^2	V_{max}, ML/s	a/P_o	Peak Power Output, μW/mg	Peak Power Output, P/P_o·ML/s	F_{opt}, P/P_o
Control (n = 43)	22 ± 9	0.87 ± 0.41	0.20 ± 0.08	1.80 ± 0.21	0.087 ± 0.025	0.28 ± 0.05
Trained (n = 46)	24 ± 8	1.41 ± 0.46	0.36 ± 0.09*	2.35 ± 0.29*	0.151 ± 0.042*	0.36 ± 0.06*

Values are means ± SD; n, no. of cells. V_{max}, maximal unloaded shortening velocity; ML, muscle length (measured after attachment); a/P_o, measure of the curvature of force-velocity relationship; P/P_o, force relative to isometric force; F_{opt}, relative force at which power output was optimal. *Significantly different from control ($P < 0.05$).

a V_{max} of 1.12 muscle lengths (ML)/s, and an a/P_o value of 0.23. Data from trained myocytes yielded a V_{max} of 1.43 ML/s and an a/P_o of 0.33.

A power-load curve was constructed by multiplying, in each cell, the velocity values times the force values for each force clamp. The resulting power output data was then summed for all trained cells and all control cells. Figure 3*B* shows the resulting mean (±SD) values for power output plotted against the relative force values for trained and control myocytes. By this analysis, peak power was 0.16 P/P_o·ML/s in trained myocytes and 0.10 P/P_o·ML/s in control myocytes in trained myocytes and 0.10 in control myocytes. The F_{opt} was 0.331 (relative tension) in trained and 0.303 in control cells. Thus the two different methods of analysis yielded similar results. Force-velocity and power output values were similar to those obtained in other studies using permeabilized myocytes (13, 14, 18).

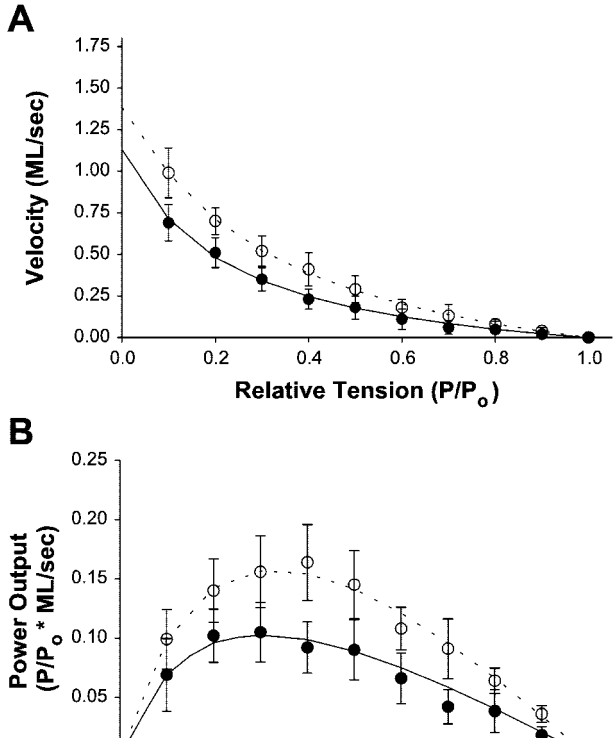

Fig. 3. *A*: composite force-velocity curves for control and trained myocytes. Data were compiled from 43 control and 46 trained myocytes. Isotonic shortening velocity data at each load were averaged from all myocytes in each group (trained vs. control). Lines are the best-fit regression line using the Hill equation as described in METHODS. Data points are presented as means ± SD. ○, Trained; ●, control. *B*: force-power curve constructed from force-velocity data. In each myocyte at each load, force values (P/P_o) were multiplied times mean velocity values (ML/s) to result in a value of power output for that load. Data points are means ± SD for all trained cells and all control cells. Lines are data from best-fit regression lines using the Hill equation. Peak power output was taken from the highest point in the best-fit line. ○, Trained; ●, control.

Figure 4 shows the results of SDS-PAGE analysis of homogenates from ventricular tissue from control and exercise-trained hearts. *Lanes 1* and *2* are representative samples taken from control hearts; *lanes 3* and *4* are representative samples from trained hearts. The *inset* shows the densitometric scan of *lanes 2* and *3*. We examined MHC isoform content of ventricular homogenates from each of the trained and control animals in the study. In the control samples (n = 7), the mean (±SD) ratio of α-MHC/β-MHC was 82:18 ± 3%. In the trained samples (n = 7), this mean ratio was 85:15 ± 4%. There was no statistically significant difference between the groups ($P < 0.05$).

DISCUSSION

Effects of exercise on force-velocity properties and power output. The primary finding of this study is the effect of exercise training to alter the force-velocity and power output properties in single cardiac myocytes. We found no effect of training on V_{max}, the maximal velocity of unloaded shortening. On the other hand, even in the absence of changes in unloaded shortening, we found that training did increase the velocity of loaded

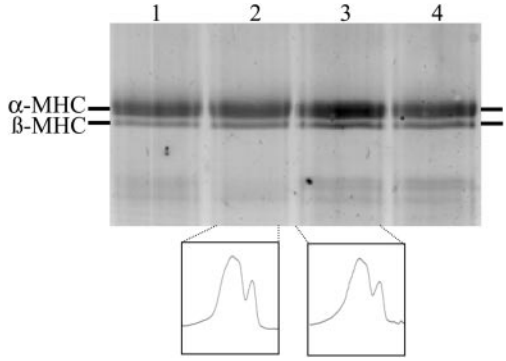

Fig. 4. Representative 6% SDS-polyacrylamide gels showing the distribution of myosin heavy chain (MHC) isoforms in ventricular homogenates from control and trained rats. *Lanes 1* and *2* are from control samples, and *lanes 3* and *4* are from trained samples. *Inset*: densitometric scans of *lanes 2* and *3*. We found no significant differences in MHC isoform content in trained samples compared with control samples.

shortening, which resulted in a decreased curvature of the force-velocity curve (higher value for a/P_o) and an increase in peak power output. This is the first direct study of the effect of training on force-velocity properties and power output in single myocytes, and these results provide evidence that exercise training increases the capacity of the myocardium to perform external work.

A number of studies using intact hearts or isolated working heart preparations have used training-induced increases in stroke volume, increases in peak pressure, or increases in the rate of pressure development as indexes of increased myocardial contractile function (2, 4, 11, 16). However, these various functional parameters may all involve different regulatory mechanisms at the cellular level. Similarly, studies using isolated myocardial preparations or isolated single cells have often used training-induced increases in isometric tension properties or shortening properties to infer a change in contractile function that supports an increase in the pumping capacity of the ventricle (6, 8, 21, 24, 35, 36). However, much of the cardiac cycle involves the ventricle shortening against a load, and the properties that regulate unloaded shortening, isometric tension development, and loaded shortening may adapt differently to exercise training. Isometric tension is ultimately limited by the number of active cross bridges and so is affected by myocyte cross-sectional area or extent of activation. Unloaded shortening velocity, on the other hand, is governed by rate of ATP hydrolysis,specifically limited by the rate of ADP release (32). Loaded shortening and hence power output is governed by some combination of these two parameters (i.e., number of cross bridges and rate of cross-bridge cycling).

Training-induced increases in isometric tension properties can likely be explained on the basis of either increased cell width (21, 24) or increases in the extent of activation either through increased Ca^{2+} sensitivity of tension (8, 36) or increased length dependence of tension (6, 24). There is significant controversy regarding the effect of training on myosin ATPase activity (reviewed in Ref. 20), but a number of studies using intact single myocytes have shown no effect of training on rate of myocyte shortening during activation (17, 21, 24, 26). The results of the present study are in agreement with these previous findings since we saw no significant effect of training on unloaded shortening (V_{max}). Because, in these experiments, V_{max} can only be obtained by extrapolation from loaded shortening values, it is highly sensitive to small measurement errors, particularly at low forces. Thus the lack of significant effect may be due in part to the significant variation in this value among myocytes. However, our results show that shortening during loaded contractions was increased in response to training, as evidenced by a significant change in the curvature of the force-velocity curve (a/P_o). The consequences of this change in curvature can be best seen in the substantially altered force-power curve (Fig. 3*B*). Peak power output was increased significantly (60% greater) in trained myocytes compared with control. Power output was increased despite the fact that maximal tension was not significantly increased in trained cells compared with control. In addition, variations in maximal tension were accounted for by expressing peak power output normalized to P_o. Both normalized peak power output and absolute peak power output were significantly increased in trained compared with control (Table 2). These results indicate that even in the absence of evidence for increased maximal velocity of shortening, exercise training induces adaptations that can increase the work capacity of the myocardium. Because muscles in vivo generally operate at intermediate forces and velocities where power is close to maximum (27) rather than at zero load or zero shortening, it is likely that adaptation of loaded shortening properties may have more relevance with respect to in vivo myocardial function.

Possible mechanisms of altered force-velocity properties. As mentioned above, shortening velocity of striated muscle is closely linked with myosin ATPase activity. One of the predominant determinants of myosin ATPase activity is the isoform of myosin that is present. There are two MHC isoforms expressed in the vertebrate myocardium, α-MHC and β-MHC. In the adult rat, the α-MHC isoform is thought to predominate (34), but the relative distribution of these two isoforms changes in response to development, hormone levels, and disease. Previous studies have indicated that alterations in MHC isoform content have significant effects on the force-velocity relationship and power output properties of single cardiac myocytes (13, 14). For this reason, we examined the MHC isoform distribution in trained and control myocardial tissue from which myocytes had been isolated. Studies examining the effect of endurance exercise training on MHC expression in the heart have yielded conflicting results. A number of studies using swimming as a training modality have suggested that exercise training induces an increase in α-MHC expression in the rat heart (25, 28), although an increase in α-MHC expression has also been observed in rats trained by running (16). A number of studies using treadmill training have found no evidence for a change in MHC expression in response to exercise training (9, 35), and a training-induced increase in β-MHC expression has been described (11). In the present study, we found no difference in the MHC isoform content in the hearts of trained animals compared with control animals. This result suggests that other factors are likely responsible for the training-induced increase in loaded shortening velocity and power output observed in the present study.

We recently reported results of a study using an identical exercise-training program indicating that training increases the expression of the atrial isoform of myosin light chain 1 ($aMLC_1$) in ventricular myocardium (7). The adult rat heart normally expresses two isoforms of the essential light chain: $aMLC_1$ in atrial tissue and $vMLC_1$ in ventricular tissue, but this pattern of MLC_1 expression in ventricular tissue has previously been shown to change under pathological conditions. In both human hypertrophic cardiomyopathy

(22) and in a porcine model of hypertension (23), $aMLC_1$ expression was shown to be increased in ventricular myocardium. This increase in $aMLC_1$ expression was well correlated to increases in the shortening velocity of a myocardial preparation (23). In addition, increased expression of $aMLC_1$ in ventricular tissue in a transgenic mouse model has been associated with an increase in myocardial shortening velocity and power output (29). The results of these studies, along with our laboratory's previous finding of an increase in $aMLC_1$ expression with exercise training (7), suggest that the increase in loaded shortening velocity and power output characteristics seen in the present study may be due, in part, to a training-induced increase in $aMLC_1$ expression in ventricular tissue.

In conclusion, we have demonstrated that an endurance-exercise training program alters the force-velocity and power output properties of rat cardiac myocytes. These alterations are characterized by an increased velocity of shortening under load and an increase in peak power output but no change in V_{max}. The mechanism for these changes is not known at present but may be related to training-induced changes in MLC isoform content.

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