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Regional differences in effects of exercise training on contractile and biochemical properties of rat cardiac myocytes

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Diffee, Gary M., and Daniel F. Nagle. Regional differences in effects of exercise training on contractile and biochemical properties of rat cardiac myocytes. *J Appl Physiol* 95: 35–42, 2003. First published January 24, 2003; 10.1152/japplphysiol.00951.2002.—Myocardial function is enhanced by endurance exercise training, but the cellular mechanisms underlying this improved function remain unclear. A number of studies have shown that the characteristics of cardiac myocytes vary across the width of the ventricular wall. We have previously shown that endurance exercise training alters the Ca^{2+} sensitivity of tension as well as contractile protein isoform expression in rat cardiac myocytes. We tested the hypothesis that these effects of training are not uniform across the ventricular wall but are more pronounced in the subendocardial (Endo) region of the myocardium. 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 the Endo region of the myocardium and from the subepicardial (Epi) region of both T and C hearts. We found an increase in the Ca^{2+} sensitivity of tension in T cells compared with C cells, but this difference was larger in the Endo cells than in the Epi cells. In addition, we found a training-induced increase in atrial myosin light chain 1 ($aMLC_1$) expression that was larger in the Endo compared with Epi samples. We conclude that effects of exercise training on myocyte contractile and biochemical properties are greater in myocytes from the Endo region of the myocardium than those from the Epi region. In addition, these results provide evidence that the increase in $aMLC_1$ expression may be responsible for some of the training-induced increase in myocyte Ca^{2+} sensitivity of tension.

atrial myosin light chain 1; chronic endurance exercise training

CHRONIC ENDURANCE EXERCISE TRAINING has been shown to enhance cardiac function as evidenced by greater maximal cardiac output and greater maximal and submaximal stroke volume (4, 7, 14, 16, 29, 30). One potential mechanism for the improved cardiac function is a training-induced enhancement of the intrinsic contractile function of the myocardium. A number of studies have provided evidence of increased contractile function on the basis of measurements in myocardial muscle preparations (18, 35, 36) or single cardiac cells (9, 20, 25, 38), although these effects are not universally observed (15, 26). Several of these studies have attempted to isolate the cellular/molecular mechanism(s) for this enhancement of contractile function. However, the results of these studies have yet to provide a clear picture of the effect of exercise training on various subcellular processes involved in the regulation of contraction (reviewed in Ref. 19). Thus there is the need for further determination of possible cellular mechanisms for training-induced improvements in contractile function.

A number of recent studies (9, 15, 20, 24, 25, 38) have focused on the effect of exercise on the morphological, electrical, and mechanical properties of single cardiac myocytes. Unfortunately, the results of these studies are also conflicting in some cases. Exercise training has been shown to result in an increased extent of shortening in electrically stimulated intact myocytes (20, 38), but this effect has not been seen in other studies (15, 25, 26). We have previously shown that exercise training increased the Ca^{2+} sensitivity of tension in rat skinned cardiac myocytes, which resulted in greater tension at submaximal Ca^{2+} levels in myocytes from trained animals than in control myocytes at the same Ca^{2+} concentration ($[Ca^{2+}]$) (9). A similar effect of training was also reported in intact myocytes (38). However, no effect of training on Ca^{2+} sensitivity was observed in other studies using skinned trabeculae (26) and intact myocytes (25).

Although some of these conflicting results may be explained on the basis of differences in training regimens or differences in experimental conditions, it is also possible that different results are due to nonuniformity of myocyte contractile and biochemical properties within the myocardium. Most studies of adaptation to training in the myocardium use tissue or cells drawn from the entire ventricular wall. However, it is clear that there are a number of regional differences in myocardial properties. Electrical properties are known to vary across different regions of the ventricle and even across the width of the ventricular wall within the same region (1). Mechanical and biochemical properties have also been shown to be different in the subendocardial (Endo) region of the myocardium compared with the subepicardial (Epi) region (5, 6). Several studies have recently pointed out that the Endo region of

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the myocardium is more responsive to adaptation to a number of stimuli, including exercise training (2, 24, 25, 31, 34). Understanding of the nature and extent of regional differences in adaptation of the myocardium to exercise training may be helpful in determining mechanisms underlying the adaptive response since regional differences in myocardial properties may relate to transmural variations in wall stress (33).

We have recently determined that exercise training results in an increase in the atrial isoform of myosin light chain 1 ($aMLC_1$) in ventricular tissue (8). This increased $aMLC_1$ expression may provide a molecular mechanism for a number of the changes in contractile properties that have been previously observed (9, 38). The purpose of this study was to test the hypothesis that there are regional differences (Epi vs. Endo) in the effect of training on $aMLC_1$ expression and that these differences lead to regional differences in training-induced alteration of contractile properties of single permeablized myocytes.

METHODS

Exercise training protocol. Female Sprague-Dawley rats were randomly divided into a control group ($n = 8$) and a training group ($n = 8$). The animals were housed in individual cages on a 12:12-h light-dark cycle and had access to food and water ad libitum. The training consisted of an 11-wk treadmill training protocol that had been shown previously to increase maximal oxygen uptake and increase cardiac performance at the whole heart (11), myocardial (35, 36), and single cell level (9). The rats were trained on a rodent treadmill starting at 15 min/day at a speed of 10 m/min and at 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 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 (9). The 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 and trimmed of atria, connective tissue, and vascular tissue. The free wall of the left ventricle was then placed on an ice-cold aluminum block and separated into Endo region and Epi region with a razor blade. These sections were quick frozen in liquid nitrogen and stored at −80°C until used to prepare myocytes for contractile measurements or used for gel analysis. On the day of an experiment, one piece of tissue 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.

Experimental apparatus. The experimental apparatus has been described previously (9). 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 a ~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. Sarcomere length was set to 2.35 μm for all cells. Myocyte length 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 by using custom software in LABVIEW for Windows (National Instruments). The experimental chamber contained three wells into which the myocyte could be moved 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), various free [Ca^{2+}] between 10^{-9} and $10^{-4.5}$ M (maximally activating 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).

Tension-Ca pH relationships. Tension was measured as a function of pCa (−log [Ca^{2+}]) in the pCa range of 9.0 to 4.5, as previously described (9). Tension was measured first in pCa 4.5 solution and then in randomly ordered submaximal pCa solutions, with every third activation made in pCa 4.5 to assess any decline in myocyte performance. For each activation, steady-state tension was allowed to develop, at which point the cell was slacked by 20% of its initial length and transferred to pCa 9.0 solution. Total tension was calculated as the difference between steady-state tension and the tension baseline immediately after the preparation was slacked. Active tension was determined by subtracting passive tension in pCa 9.0 from total tension. Maximal tension (in pCa 4.5 solution) was determined and normalized for the cross-sectional area of the cell. Cross-sectional area was calculated from the diameter of the cell and by assuming a circular cross section. Tension at each pCa was expressed as a percentage of maximal tension for that preparation. Data were analyzed by least-squares regression by using the Hill equation as described previously (9). Analysis using the Hill equation yielded values for the the pCa giving half-maximal tension (pCa_{50}), which was used as an index of Ca^{2+} sensitivity of tension.

Two-dimensional gel electrophoresis. To determine the effect of training on $aMLC_1$ protein levels in Endo and Epi regions, we analyzed homogenates from control and trained heart samples by using two-dimensional (2D) gel electrophoresis. Frozen tissue was homogenized at 100 mg/ml in sample buffer (8 M urea, 2 M thiourea, 75 mM DTT, and 10 mM Tris, pH 7.0) by using a Fisher PowerGen 700 homogenizer with a 7-mm saw-tooth generator at 20,000 rpm for 8–10 s. The protein concentration of this homogenate was

determined by using a Bio-Rad protein assay kit with bovine serum albumin as the standard. Isoelectric focusing (IEF) was performed by using Bio-Rad's Protean IEF cell and 11-cm precast IPG gel strips (pH 3–10). Three hundred micrograms of protein in sample buffer plus 1.85% CHAPS and 0.185% carrier ampholytes were loaded onto the strips via 1 h of passive rehydration and 12 h of active loading at 50 V and 20°C. The Bio-Rad IEF unit was programmed to rapidly ramp to 250 V for the first 15 min, rapidly ramp to 6,700–7,000 V for the next 2.5 h (limited to 50 mA/strip), and hold at peak voltage and 50 mA/strip for 35,000 V-h. Strips were held at 500 V at the conclusion of their run until removed from the power unit. Strips were incubated in equilibration *buffers I* (125 mM Tris·HCl, pH 6.8, 20% glycerol, 2% SDS, 6 M urea, 2% DTT) and *II* (Tris·HCl, pH 6.8, 20% glycerol, 2% SDS, 6 M urea, 2.5% iodoacetamide) for 20 min each. After equilibration of the strips, second dimension PAGE was performed by using 12.5% Bio-Rad Criterion precast gels with IPG + 1-well combs, run at 20 mA/gel for 45 min and 30 mA/gel for the duration of the run (2.5 h total). Gels were stained by using a zinc stain (Pierce) and digitized by using a Kodak image station 440CF. Sixteen exposures were summed to increase signal-to-noise ratio. PDQuest software (Bio-Rad, version 6.0) was used for gel image analysis. The locations of the $aMLC_1$ and ventricular MLC_1 ($vMLC_1$) spots were determined on the basis of the predicted isoelectric point (pI) and molecular ratio of these proteins as well as by comparison to previously published 2D gel analysis of these proteins (21, 27).

Mass spectrometry. To confirm the identity of the $aMLC_1$ spot, the spot was excised from the gel, digested with trypsin, and the peptides analyzed by MALDI-TOF mass spectrometry at the University of Wisconsin-Madison Biotechnology Center with a Bruker Biflex MALDI-TOF MS mass spectrometer (Bruker Daltonics, Billerica, MA.) as described in Ref. 12. The results of this analysis confirmed the identity of the spot as $aMLC_1$.

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 (32).

Statistical analysis. Between-group comparisons (trained vs. control) were made by using Student's *t*-test, with $P < 0.05$ considered to indicate a statistically significant difference. To determine differences between groups (trained vs. control) or between regions (Endo vs. Epi) in Ca^{2+} sensitivity, pCa_{50} data was determined for each cell individually. These data were pooled for a minimum of 10 cells from each animal (5 from each region), producing a mean pCa_{50} for each region of each animal. These data were then pooled to produce a mean pCa_{50} for each region in trained ($n = 8$ animals) and control ($n = 8$ animals) groups. Differences between Epi and Endo cells from control and trained animals were analyzed by two-way ANOVA followed by Student's *t*-test. The data presented in Fig. 1 represent mean values for all trained cells from a given region (Endo and Epi) and all control cells from a given region.

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 data were all significantly different in trained compared with control animals ($P < 0.05$).

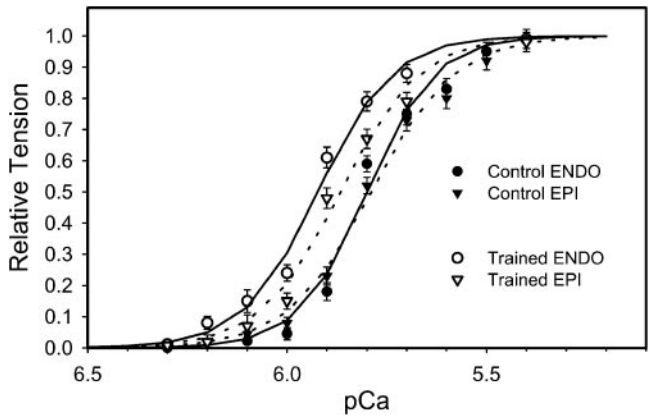

Fig. 1. Composite tension-pCa curves for control and trained myocytes from different regions of the myocardium. Data were compiled from 50 control and 50 trained myocytes [25 cells from subendocardial (Endo) and 25 cells from subepithelial (Epi) regions in each group]. Relative tension data at each pCa were averaged from all myocytes in each group. Data points are presented as means ± SD. Lines are the best fit regression line using the Hill equation as described in METHODS. The pCa giving half-maximal tension (pCa_{50}) values given for each group were control Epi (▼) = 5.79, control Endo (●) = 5.80, trained Epi (▽) = 5.87, and trained Endo (○) = 5.92.

Table 2 gives the characteristics of myocytes isolated from the Epi region and Endo region of hearts from trained and control animals. We found that there were no significant regional differences in cell width, passive tension (measured in pCa 9.0 solution), or maximal tension (measured in pCa 4.5 solution). In addition, there were no significant effects of training on these myocyte characteristics.

Tension-pCa relationship. Figure 1 shows the relationship between tension and $[Ca^{2+}]$ (i.e., pCa) in the four populations of myocytes in this study. This figure was constructed by plotting, for each pCa tested, the mean relative tension (expressed as a fraction of the maximal tension for that cell) data for all of the myocytes in each group ($n = 40$ myocytes/group). Each group of data was then fit to the Hill equation (best fit is shown by lines in Fig. 3), which resulted in a value for the pCa_{50}. The data in Fig. 1 reveal that there was no difference in Ca^{2+} sensitivity of tension between Epi and Endo myocytes from control hearts. The pCa_{50} for control Epi myocytes was 5.79, whereas the pCa_{50} for control Endo myocytes was 5.80. Training caused an increase in Ca^{2+} sensitivity of tension, as evidenced by a left shift in the tension-pCa relationship. The increase was greater in the trained Endo cells ($pCa_{50} = 5.92$) than in trained Epi cells ($pCa_{50} = 5.87$).

Figure 2 presents another method of analysis of the effects of training and region on Ca^{2+} sensitivity that allows statistical comparison between groups. For this

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

	Control	Trained
Prebody wt, g	200.1 ± 6.9	198.4 ± 8.6
Postbody 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, $\mu mol \cdot min^{-1} \cdot g$ wet wt^{-1}	15.07 ± 0.59	21.44 ± 1.68*

Values are means ± SD. Pre, before exercise; post, after exercise. *Significantly different from control ($P < 0.05$).

analysis, force data for each myocyte were analyzed by using the Hill equation, which resulted in a pCa_{50} value for each cell. The mean pCa_{50} values were then determined from all cells from a given region (Epi vs. Endo) in a given animal ($n = 10$ cells/animal). Finally, mean pCa_{50} values were determined for each region and each group (trained vs. control) from $n = 8$ animals/group. These data are presented in Fig. 2. We found a significant effect of training on pCa_{50} values ($P < 0.05$) and also found that Endo cells had a significantly greater increase ($P < 0.05$) in pCa_{50} compared with control than did Epi cells.

2D electrophoresis analysis. To determine changes in expression of $aMLC_1$, we performed 2D electrophoretic analysis of homogenates from the four groups of tissue from which myocytes had been isolated. This method has been used previously to separate the ventricular and atrial isoforms of MLC_1 in human (21, 27), porcine (22), and rat (8) myocardium. A representative 2D gel is shown in Fig. 3*A*. The highlighted area was analyzed for the presence of $aMLC_1$ and $vMLC_1$ based on the predicted pI and molecular weight (MW) of these two isoforms. A magnified image of this region of the gel is shown in Fig. 3*B*. Identification of spots corresponding to $aMLC_1$ and $vMLC_1$ was based on pI and MW information as well as previously published determinations of $aMLC_1$ vs. $vMLC_1$ positions on 2D gels (22, 27). The predicted pI and MW of rat aMLC1 is 4.97 and 21,150.99 respectively, whereas the pI and MW of $vMLC_1$ is 5.03 and 22,025.01 (http://us.expasy.org). The identity of the $aMLC_1$ spot was confirmed by using

Table 2. *Characteristics for single cardiac myocytes isolated from different regions of control and trained hearts*

	Cell Width, μm	Passive Force, μN	Passive Force, kN/m²	Maximal Force, kN/m²
		Control		
Endo	24 ± 5.6	0.71 ± 0.29	1.5 ± 0.7	21.6 ± 5.4
Epi	26 ± 3.3	0.77 ± 0.21	1.4 ± 0.6	19.3 ± 5.7
		Trained		
Endo	31 ± 4.4	0.79 ± 0.28	1.7 ± 0.6	21.7 ± 6.8
Epi	28 ± 5.2	0.84 ± 0.37	1.8 ± 0.7	22.5 ± 5.2

Values are means ± SE. Endo, cells taken from subendocardial region of the myocardium; Epi, cells taken from subepicardial region of the myocardium; Cell width, width of skinned cells attached to apparatus and set to sarcomere length of 2.35 μm.

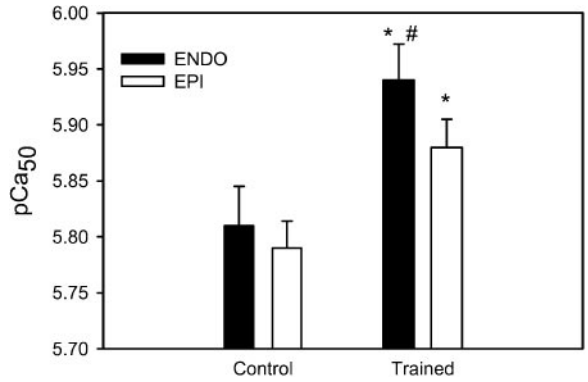

Fig. 2. Effect of training and myocardial region on Ca^{2+} sensitivity of tension in single cardiac myocytes. Mean pCa_{50} values in myocytes from Endo (black bars) and Epi (open bars) regions of the myocardium from trained and control hearts ($n = 8$ animals per group) are shown. *Significantly different from control ($P < 0.05$). #Significantly different from Epi ($P < 0.05$).

mass spectrometry. Densities of spots corresponding to $aMLC_1$ and $vMLC_1$ were quantified by using PDQuest software, and the amount of each MLC_1 was expressed as a percentage of the total MLC_1 in that sample. We performed 2D gel analysis on $n = 6$ samples from each of the four groups (control Epi and Endo and trained Epi and Endo). Mean data show that there was no detectable $aMLC_1$ protein in either of the control samples (Epi or Endo). In trained animals, $aMLC_1$ content was 17.2 ± 3.2% of the total MLC_1 in Endo samples and 8.4 ± 2.8% of the total MLC_1 in Epi samples. The $aMLC_1$ content in trained samples was significantly different from control ($P < 0.05$). There was also a significant interaction between training and region (Epi vs. Endo) in $aMLC_1$ content ($P < 0.05$).

To determine the degree of correlation between changes in Ca^{2+} sensitivity and changes in $aMLC_1$ expression, we plotted the $aMLC_1$ content [expressed as $aMLC_1/(aMLC_1 + vMLC_1)$] of each of the samples vs. the mean pCa_{50} data from myocytes taken from those samples. These data are shown in Fig. 4. The pCa_{50} data represent mean data from all of the myocytes from a given region (Epi vs. Endo) from a given animal (e.g., C1, T2, etc.). The plot shows a significant positive correlation between $aMLC_1$ content and pCa_{50} irrespective of the training state of the animal or regional origin of the tissue. The regression line has a correlation coefficient of $r^2 = 0.72$ with $P < 0.05$.

DISCUSSION

The primary findings of this study are that the effects of exercise training on contractile protein expression and on contractile properties of cardiac myocytes vary across the width of the ventricular wall. This result emphasizes the diversity of myocyte characteristics and adaptability across the myocardium and suggests that the stimulus for adaptation to exercise training may not be uniform across different regions of the myocardium.

Regional differences in control myocardium. Previous studies have determined that there are significant

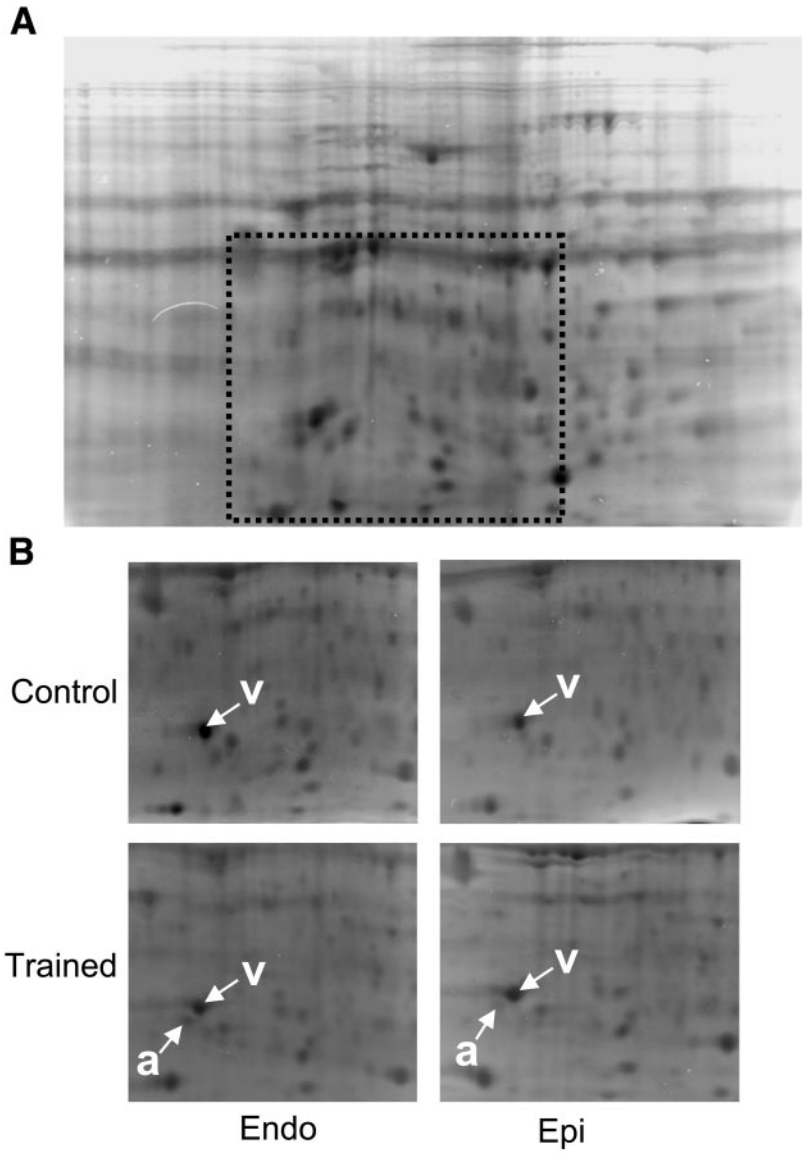

Fig. 3. Representative sample of 2-dimensional (2D) electrophoresis analysis of regional differences in atrial myosin light-chain 1 ($aMLC_1$) protein expression in trained and control ventricular tissue. *A*: representative 2D gel showing results of first dimension IEF using a pH 3–10 gradient and second dimension SDS-PAGE with 12.5% acrylamide gel. This sample was a mixture of atria and ventricular homogenates to show the position of $aMLC_1$ and ventricular myosin light chain 1 ($vMLC_1$). *B*: close-up of $aMLC_1$-$vMLC_1$ region of gels (box in *A*) used for analysis. Shown are representative gels using homogenates from control and trained Epi and Endo tissue. Identification of $aMLC_1$ (a) and $vMLC_1$ (v) is based on predicted isoelectric point (pI) and molecular weight (MW) values for these proteins as well as previously published 2D electrophoretic analyses of these proteins and confirmed by mass spectroscopy. In these samples, there was no $aMLC_1$ detected in either of the control samples. In the trained Endo sample shown, $aMLC_1$ comprised 16% of the total myosin light chain 1 pool. The $aMLC_1$ content in the trained Epi sample shown was 5% of the total myosin light chain 1.

regional differences in the electrical, mechanical, and morphological properties of the ventricular myocardium. Endo cells have been reported to have an increased width and increased volume compared with Epi myocytes (25), although these differences have not been seen in all studies (6, 24). In addition, both passive tension (6) and active maximal tension (6, 25) have been shown to be greater in Endo cells compared with Epi cells. In the present study, we found no evidence of regional differences in control cells in any of the myocyte characteristics that we measured, including cell size and passive and active tension properties. It is important to note that previous measurements of regional differences in cell mechanical properties were made by using intact, electrically stimulated myocytes (6, 23, 24) compared with the permeablized preparations used in the present study. Because permeabilization results in the disruption of the sarcolemma as well as subcellular organelles, our result suggests that regional differences between myocytes in control myocar-

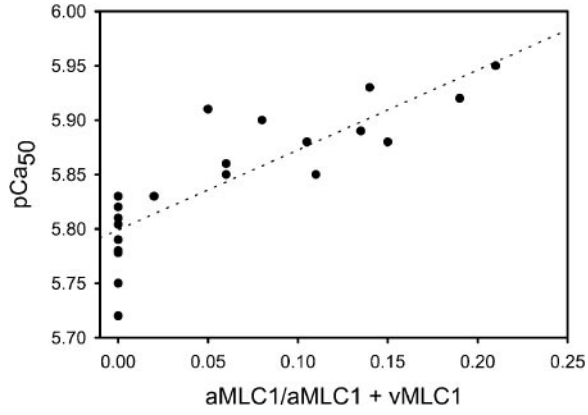

Fig. 4. Correlation between pCa_{50} of cardiac myocytes and the $aMLC_1$ content (fraction of total MLC_1) of the tissue from which the myocytes were isolated. pCa_{50} values are means from a minimum of 5 cells/group. The slope of the regression line is 0.74. The correlation coefficient is 0.72, which represents a significant correlation ($P < 0.01$).

dium may depend on cell processes or properties that are lost on permeabilization. However, our observation of regional differences in permeablized cell properties in trained myocardium (discussed in *Regional differences in response to training* below) suggests that these training-induced changes are localized within the contractile element.

Regional differences in response to training. In addition to regional differences in Endo vs. Epi cells outlined above, there is substantial evidence to indicate that these different regions of the myocardium also respond differently to a variety of stressors (2). Action potential properties have been found to respond differently to hypertrophic stimuli in Endo compared with Epi myocytes (5, 31). Pressure overload is known to increase β-myosin heavy chain (MHC) expression, and this increase was found to be greater in endocardial cells compared with epicardial cells (34). Exercise training has been shown to have a greater effect on myocyte characteristics, including cell size and length-tension properties, in Endo myocytes than in Epi myocytes (24, 25).

We found no effect of training on cell width, a result consistent with what we have observed previously (9). However, because the permeabilization process can affect cell width, these measurements in skinned cells may not be an accurate method to determine the effect of training on cell width. Other studies have also found no effect of training on cell width (20, 38), but some studies have found that training significantly increases cell width, and this increase is greater in cells from the Endo region (24, 25). We found no effect of training or region on passive tension or maximal tension. We have previously found that exercise training induces an increase in tension at any given submaximal [Ca^{2+}] in single myocytes (9). This increased sensitivity to activation by Ca^{2+} has been observed in other (38) but not all (25, 26) studies. With an exception (25), all of these previous experiments were done by using cell populations taken from the entire ventricular wall. The results of the present study indicate that these changes in Ca^{2+} sensitivity are not distributed across the ventricular wall uniformly. We again found a significant effect of training on Ca^{2+} sensitivity of tension (as measured by pCa_{50}) but found that Ca^{2+} sensitivity was increased to a greater extent in trained Endo myocytes than in trained Epi myocytes. We had previously observed a training-induced leftward shift in the pCa_{50} (indicative of increased Ca^{2+} sensitivity) of 0.08 pCa units in a mixed population of cells (9). We found an average shift of 0.14 pCa units in Endo cells from trained hearts compared with control Endo cells, whereas trained Epi cells were shifted an average of 0.07 pCa units compared with control Epi cells. In both the present study as well as our laboratory's earlier study (9), the absolute value of [Ca^{2+}] at which tension is measured differs from the [Ca^{2+}] in studies that used intact myocyte or trebeculae preparations (26, 38). These differences are likely due to changes in activation properties associated with permeabilazation, different experimental temperatures, and other methodological differences. The assumption is that training-induced changes or regional differences in Ca^{2+} sensitivity observed in permeablized preparations persist in intact preparations even though the absolute [Ca^{2+}] might differ between these preparations.

We have also previously identified a training-induced increase in the expression of $aMLC_1$ in ventricular tissue (8). In the present study, we again found a training-induced increase in $aMLC_1$ expression and determined that this increase in expression varies across the width of the myocardium. We found no evidence for expression of $aMLC_1$ in homogenates from control tissue regardless of region. We found that the $aMLC_1$ content (expressed as a fraction of total MLC_1 in the sample) in homogenates from the Endo region of the myocardium was significantly greater than that found in the Epi region, although $aMLC_1$ content in both regions was significantly greater than control.

Effect of $aMLC_1$ on Ca^{2+} sensitivity. The variability of $aMLC_1$ content across different regions of the myocardium, as well as variability between animals, provides an opportunity to correlate altered $aMLC_1$ expression with altered function of the myocardium. Increases in $aMLC_1$ content in human hypertrophic cardiomyopathy (21) and in a porcine model of hypertension (22) have been correlated with increased Ca^{2+} sensitivity of tension (21) and increased maximal shortening velocity (22) in these models. In a transgenic mouse model in which $aMLC_1$ was overexpressed in ventricular tissue, shortening velocity and power output were found to be increased (28). In the present study, we found that a positive correlation exists between variations in $aMLC_1$ content and variations in Ca^{2+} sensitivity (Fig. 4). There are a number of potential regulators of Ca^{2+} sensitivity in cardiac myocytes, and the variability in pCa_{50} values in samples in which no $aMLC_1$ was detected indicates that there are clearly other sources of variability present in our measurements. However, the existence of a significant correlation between these two variables suggests that at least some of the training-induced increase in Ca^{2+} sensitivity of tension in myocytes can be attributed to a training-induced increase in $aMLC_1$ expression.

The mechanism(s) for the effects of increased $aMLC_1$ expression to alter myocardial contractility is not known. The mechanism(s) is thought to be related to sequence differences between $aMLC_1$ and $vMLC_1$ in the NH_2-terminal region of the light chain (21). There is evidence that this region of the MLC_1 molecule interacts with the actin filament during cross-bridge formation (37).

The regional differences in the contractile and biochemical response to exercise training may suggest possible mechanisms for alteration in $aMLC_1$ expression by training. Previous studies have indicated that pressure overload of the ventricle increases the expression of β-MHC in cardiac myocytes (13, 17, 23). Swoap et al. (34) found that, although β-MHC expression was greater in Endo cells compared with those from the Epi region even in control hearts, pressure overload in-

creased this difference. Presumably this gradient of MHC isoform expression in control hearts and in pressure overload is related to the pressure gradient that exists across the ventricular wall (33). Expression of $aMLC_1$ is also increased under conditions of pressure overload (22), suggesting that regional differences in training-induced increases in $aMLC_1$ expression might also be related to pressure gradients across the ventricular wall. However, it is not known whether *1*) the mechanism(s) involved in increased $aMLC_1$ expression as a result of exercise training is the same as that involved in the response to pressure overload or *2*) there is a relationship between transmural pressure gradients in the myocardium and regional differences in the myocardial response to exercise training.

In conclusion, we have provided evidence that training-induced increases in myocyte Ca^{2+} sensitivity of tension are more pronounced in myocytes from the Endo region of the myocardium compared with those from the Epi region. In addition, we have demonstrated that training-induced increases in the expression of $aMLC_1$ are also more pronounced in the Endo region of the myocardium compared with the Epi region. The positive correlation that we observed between increases in myocardial $aMLC_1$ expression and increases in myocyte Ca^{2+} sensitivity suggests that the training-induced changes in $aMLC_1$ expression may provide a possible molecular mechanism for the effect of training to alter contractile properties in the ventricular myocardium.

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