Isometric contraction induces rapid myocyte remodeling in cultured rat right ventricular papillary muscles

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Guterl KA, Haggart CR, Janssen PM, Holmes JW. Isometric contraction induces rapid myocyte remodeling in cultured rat right ventricular papillary muscles. Am J Physiol Heart Circ Physiol 293: H3707–H3712, 2007. First published October 5, 2007; doi:10.1152/ajpheart.00296.2007.—The hypothesis that elevated systolic stress induces myocyte thickening has been difficult to test directly. We tested this hypothesis in working rat ventricular papillary muscles using a recently developed technique for long-term muscle culture. Muscles were cultured for 36 h either isometrically at different levels of systolic stress or at physiological amounts and rates of shortening. Isometric contraction induced rapid increases in myocyte diameter regardless of the level of systolic stress, whereas control myocyte dimensions were maintained if physiological amounts and rates of systolic shortening were imposed. Myocyte thickening was accompanied by a significant decrease in cell length and number of sarcomeres in series along the long axis of the myocyte, suggesting that thickening may have occurred in part by rearrangement of existing sarcomeres. We conclude that the pattern of systolic shortening and/or diastolic lengthening regulates myocyte shape in working rat right ventricular papillary muscles, whereas systolic stress plays little or no role.

MATERIALS AND METHODS

Muscle isolation. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (11) and approved by Columbia University’s Institutional Animal Care and Use Committee. Male LBN-F1 rats (Harlan Sprague-Dawley; Indianapolis, IN), 298 ± 26 g (means ± SD), were anesthetized by intraperitoneal injection of 150 mg/kg pentobarbital sodium. After intracardiac heparinization, the hearts were rapidly dissected and perfused through the aorta with a chilled Krebs-Henseleit solution containing (in mM) 118 NaCl, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 20 NaHCO3, 11 glucose, 0.25 CaCl2, and 30 2,3-butanedione monoxime (BDM) and 20 IU/l insulin in equilibrium with 95% O2-5% CO2. One or two right ventricular papillary muscles were dissected from each rat, leaving a block of tissue at one end from the right ventricular septum and chordae tendineae at the other. The tissue block and chordae were used during handling to minimize damage to the right ventricular papillary muscle. Average dimensions of the muscles were (means ± SD) 232 ± 77 μm in major radius, 151 ± 49 μm in minor radius, and 2.00 ± 0.77 mm in length between the mounting pins. Muscles were either placed in the trabecula culture system as described in Experimental protocol or placed directly in 10% formalin for several days (control muscles), followed by dissociation and determination of myocyte dimensions and sarcomere length.

Experimental protocol. A culture of working rat right ventricular papillary muscles was performed using a modified version of the trabecula culture system described in detail by Janssen et al. (12). Muscles were mounted at slack length in the trabecula culture system between pins connected to a force transducer on one end and a programmable servomotor on the other. The perfusion solution was then exchanged for a BDM-free Krebs-Henseleit solution. Muscles were stimulated end to end at ~30% above threshold voltage (2–4 V) with 5-ms asymmetric pulses, at a frequency of 1 Hz. The calcium

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concentration of the Krebs-Henseleit solution was raised stepwise to 1.75 mM, and the solution temperature was raised to 37.0 ± 0.2°C. With the use of the micromanipulator, each muscle was stretched by 0.05-mm increments to 90–95% Lmax, the length at which maximum active force is developed. After equilibration and force stabilization, the Krebs-Henseleit solution was exchanged for a serum-free-modified cell culture medium (medium 199, Sigma) with the additions (in mM) of 2.0 t-carnitine, 5.0 creatine, 5.0 taurine, and 2.0 L-glutamine and 100 IU/ml penicillin, 0.1 mg/ml streptomycin, and 20 IU/ml insulin. Forces were recorded using a custom-designed data acquisition program (LabVIEW, National Instruments). Both medium and gas were changed at regular intervals of 7–11 h under sterile conditions.

Following prestretch and equilibration, muscles were cultured for 36 h under one of three conditions. One set of muscles contracted isometrically (n = 8). In a second set (n = 7), muscles contracted isometrically but the media was supplemented with 10 mM BDM to diminish systolic force generation (13). In a third set (n = 6), the Krebs-Henseleit solution was supplemented with 10 mM 2,3-butanedione monoxime (BDM) to isometrically but the media was supplemented with 10 mM BDM to isometrically but the media was supplemented with 10 mM BDM to diminish systolic force generation (13). In a third set (n = 6), the Krebs-Henseleit solution was supplemented with 10 mM 2,3-butanedione monoxime (BDM) to diminish systolic force generation (13). In a third set (n = 6), physiological amounts (15%) and rates (1 muscle length/s) of shortening (10) were imposed during contraction by use of the computer-controlled servomotor. Following these protocols, myocyte dimensions, sarcomere length, and the number of sarcomeres per myocyte were determined as outlined in Myocyte dimensions and sarcomere length.

Several additional experiments were performed to exclude alternative explanations for the increased myocyte diameter observed in cultured muscles. We examined the relationship between myocyte CSA and radial location in 16 muscles (8 isometric and 8 control). To exclude contributions from myocyte edema, control (n = 2) and isometrically cultured muscles (n = 2) were fixed and sent to an independent laboratory for blinded transmission electron microscopy. Finally, to examine the possible role of new protein synthesis in myocyte size changes, myocyte CSA was examined in 16 muscles cultured isometrically for 36 h with (n = 8) or without (n = 8) 100 μM puromycin, a dose shown to inhibit protein synthesis in isolated cardiac muscle preparations (19).

Myocyte dimensions and sarcomere length. After 36 h of culture, muscle dimensions were determined at diastolic length using a calibration grid in the ocular of the dissection microscope (×40; resolution, 10 μm). Muscles were then fixed at diastolic length in 10% formalin for several days. Muscle ends were removed and myocytes from the central region of each muscle were dissociated according to the KOH dissociation method of Gerdes et al. (7). Dissociated myocytes were imaged at ×400 using light microscopy. Myocyte length and width were manually determined by a blinded observer using Scion Image [National Institutes of Health (NIH)]. Matlab was used to perform a fast-fourier transform on the central region of each myocyte image to determine the average sarcomere length for a given cell. The number of sarcomeres along the long axis of the myocyte was estimated by dividing myocyte length by sarcomere length for each myocyte. An average of 113 ± 30 cells per muscle were analyzed, based on preliminary tests showing that an analysis of 100 cells limited the difference between sample mean and true population mean to <5%. Because control muscles were fixed at slack length, although cultured muscles were fixed at diastolic length, all myocyte dimensions were corrected to a sarcomere length (SL) of 2.2 μm for comparison, assuming constant myocyte volume [corrected length = L(2.2/SL); corrected diameter = D(2.2/SL)]

To examine the relationship between radial location and myocyte CSA, muscles were fixed at diastolic length in 10% formalin for several days, then embedded in glycol methacrylate (EB Sciences) and sectioned perpendicular to the muscle long axis at 6 μm thickness. Cross sections taken from the central region of the muscle were stained with Movat’s silver impregnation to highlight cell membranes and photographed at ×200 under light microscopy. With the use of Scion Image (Frederick, MD), the entire muscle outline and the outline of every myocyte in the cross section were digitized, and the CSA, centroid, distance to the nearest point on the muscle surface, and major and minor axes of a best-fit ellipse were calculated for every myocyte (>500 per section on average).

Statistical analysis. Differences among myocyte dimensions and related parameters from the four groups (control, isometric, BDM, and shortening) were evaluated statistically as follows. First, a one-way analysis of variance (ANOVA) was performed to test whether the mechanical environment had a significant effect on the dimension or parameter (InStat v3.0, GraphPad Software, San Diego, CA). If a significant effect of mechanical environment was detected by ANOVA, a Dunnett multiple comparisons test was performed to determine which groups differed significantly from control. P < 0.05 was used as the significance threshold for all tests. To determine whether a significant relationship existed between myocyte CSA and myocyte location within the muscle, linear regressions between myocyte CSA and distance from the muscle surface were performed for each muscle in GraphPad InStat and the correlation coefficient for each muscle was tested against a hypothetical mean of 0. The squared correlation coefficient and regression slopes and intercepts for control versus isometric muscles were compared using unpaired t-tests.

Fig. 1. Effect of shortening and stress reduction on myocyte dimensions. Muscles were fixed immediately after dissection (control) or cultured isometrically, isometrically with 10 mM 2,3-butanedione monoxime (BDM) to reduce force generation or with 15% imposed shortening (shortening) for 36 h. A: average peak systolic stress for each 6-h time period. Isometrically contracting muscles maintained systolic stresses over the course of the experiment; BDM reduced peak systolic stress, whereas shortening did not. B: myocyte dimensions, corrected for differences in sarcomere length between groups (see text for details). Myocyte length and length-to-diameter ratio (L/D) decreased significantly, and myocyte diameter increased significantly in isometric and BDM groups but not in shortening group. *P < 0.05 vs. control.
RESULTS

Effect of systolic stress on myocyte dimensions. Myocyte diameter increased significantly in isometrically cultured muscles, but the increase did not correlate with the level of systolic stress (Fig. 1). Myocyte dimensions were measured in four groups of muscles: isometrically contracting muscles (isometric), isometrically contracting muscles in which systolic stress was reduced by more than half by treatment with BDM (BDM), muscles that were allowed to shorten at physiological amounts and rates (shortening), and control muscles not subjected to muscle culture. Myocyte diameter increased relative to controls in both isometric (27.3 ± 15.9% increase) and BDM muscles (20.7 ± 14.4%) despite the very different systolic stresses experienced by these muscles throughout the culture period. By contrast, shortening muscles showed no significant change in myocyte dimensions despite peak systolic stress levels similar to the isometric group.

When viewed in cross section (Fig. 2), myocytes were mildly elliptical, with a major-to-minor axis ratio that was slightly lower in muscles cultured isometrically (1.63 ± 0.06) than in control muscles not subjected to muscle culture (1.77 ± 0.13). Myocyte CSA measured in these cross sections corresponded closely to CSA values estimated from isolated myocyte diameters, assuming those diameters reflected the cross-sectional major axis. There was a very weak but statistically significant correlation between myocyte CSA and distance from the muscle surface in both control ($r^2 = 0.032 \pm 0.029$; Bonferroni-corrected $P < 0.05$ in 5 of 8 muscles) and isometrically cultured muscles ($r^2 = 0.057 \pm 0.058$; Bonferroni-corrected $P < 0.05$ in 5 of 8 muscles). The trend in both muscles was for myocyte CSA to be largest at the surface of the muscle and decrease toward the center; the average regression slope was not significantly different between control ($-0.26 \pm 0.39 \mu m^2/\mu m$) and isometric ($-0.41 \pm 0.35 \mu m^2/\mu m$) muscles. Blinded transmission electron microscopy revealed a mild increase in extracellular space in the isometrically cultured muscles but no evidence of cellular edema in any group.

Contributions of growth versus rearrangement. The increase in myocyte diameters observed in both the isometric and BDM groups was accompanied by similar reductions in myocyte length (isometric, 27.4 ± 9.5% decrease; BDM, 25.8 ± 11.1%), suggesting that the observed dimension changes may have occurred in part by rearrangement of existing sarcomeres. This possibility was assessed in three ways. First, mean sarcomere length determined by Fourier transform of the isolated myocyte images was used to estimate the number of sarcomeres in series along the length of each myocyte. The number of sarcomeres per cell was reduced by more than 20% in myocytes from isometric (27.3 ± 4.4%) and BDM (26.9 ± 6.5%) muscles but was not different from control in the shortening muscles (Fig. 3). Second, myocyte volume was estimated from the measured diameters and lengths using the cross-sectional major-to-minor axis ratio measured in fixed cross sections (see Effect of systolic stress on myocyte dimensions). Estimated myocyte volume was not significantly different from control in any group (Fig. 3, $P = 0.096$), but high relative standard deviations in the estimated volumes limited the statistical power of this comparison; a power analysis showed that, at this sample size, we had only 10% power to

Fig. 2. Silver-stained cross sections of representative control and isometrically cultured muscles, showing spatially uniform changes in myocyte cross-sectional area and lack of gross cellular edema or damage. A: cross section of control rat papillary muscle fixed unloaded immediately after dissection from right ventricle. B: cross section of papillary muscle maintained for 36 h in culture and imaged at the same magnification (scale bar = 200 μm). C: same cross section as in A, magnified by digital zoom to highlight myocyte sizes. D: same cross section as in B, magnified by digital zoom to highlight myocyte sizes.
ventricular papillary muscles, whereas systolic stress plays little or no role.

Myocyte hypertrophy: size versus shape. In seeking a conceptual framework to understand the role of mechanical stimuli across the range of known experimental models of cardiac hypertrophy, we have proposed that the regulation of myocyte size and the regulation of myocyte shape should be considered separately (10). Cardiac hypertrophy is by definition an increase in myocyte size and is observed in many classical experimental models, including pressure overload and volume overload. Because our estimates of myocyte volume were too variable to test for increased myocyte size with appropriate statistical power and because we did not measure the amounts of protein or the rates of protein production and degradation in our cultured muscles, we have not referred to the dimension changes reported here as hypertrophy.

Regulation of myocyte shape. Regulation of myocyte shape is an equally important component of the process conventionally referred to as cardiac hypertrophy. Although pressure and volume overload both produce an increase in myocyte size, the resulting growth patterns are distinguished from one another by the change in myocyte shape. In pressure overload, myocytes become relatively thicker [reduced length-to-diameter ratio ($L/D$)] (2), whereas in volume overload they become relatively longer (increased $L/D$) (14, 15). Understanding the basis for these differential patterns of shape change is arguably at least as important as understanding the basis for changes in myocyte size, since one pattern of shape change (decreased $L/D$ as in pressure overload) generally results in a clinically stable configuration, whereas the other (increased $L/D$ as in volume overload) frequently leads to progressive chamber dilation and heart failure (4). The present experiments provide important new insight into the regulation of myocyte shape by mechanical stimuli.

Based on the results presented here, we propose that myocyte shape is regulated by the dynamic pattern of length changes experienced by the cell. Specifically, we hypothesize that decreased shortening leads to a decreased $L/D$ ratio and increased shortening to an increased $L/D$ ratio. In vivo, pressure overload reduces stroke volume, local myocyte shorten-

Fig. 3. Contributions of growth vs. remodeling to myocyte dimension changes. A: Fourier transforms were used to detect average sarcomere length, which was combined with myocyte length to estimate the number of sarcomeres in series along each cell. B: the number of sarcomeres ($S$) in series along each myocyte dropped significantly as myocyte width increased, suggesting rearrangement of existing sarcomeres as a possible mechanism. Computed increases in myocyte volume ($V$) were small, again suggesting that rearrangement rather than growth was the dominant process. *$P < 0.05$ vs. control.

detect a 20% change in estimated cell volume. Finally, myocyte CSA increased equally in the presence (439 ± 101 μm², $n = 8$ muscles, $P < 0.001$ vs. control) and absence (436 ± 62 μm², $n = 8$, $P < 0.001$ vs. control) of 100 μM puromycin, a dose shown to inhibit protein synthesis in isolated cardiac muscle preparations (19) (control myocyte CSA, 265 ± 62 μm², $n = 31$).

DISCUSSION

The hypothesis that elevated systolic stress induces myocyte thickening has been difficult to test directly. We tested this hypothesis in working rat right ventricular papillary muscles using a recently developed technique for long-term muscle culture (12). Isometric contraction induced rapid increases in myocyte diameter regardless of the level of systolic stress, whereas control myocyte dimensions were maintained if physiological amounts and rates of systolic shortening were imposed. Myocyte thickening was accompanied by a decrease in cell length and the number of sarcomeres in series along the long axis of the myocyte, suggesting that thickening may have occurred in part by rearrangement of existing sarcomeres. We conclude that the pattern of systolic shortening and/or diastolic lengthening regulates myocyte shape in working rat right

Fig. 4. Comparison of myocyte $L/D$ ratios measured in disaggregated fixed myocytes following in vitro culture ($\bullet$, means ± SD of values from this study) or in vivo hemodynamic overload ($\circ$, means ± SD of several literature reports). Groups are presented from left to right in order of increasing amount of systolic shortening: I, isometric contraction (2% to 3%); PO, pressure overload (Ref. 2); S, physiological shortening (15%); control, control values from this study ($\bullet$) and published reports (Refs. 2, 5, and 6) ($\circ$); VO, volume overload (Refs. 4 and 15).
ing, and \( L/D \); volume overload increases stroke volume, local myocyte shortening, and \( L/D \) (Fig. 4). By this hypothesis, isometric contraction of a working papillary muscle at sarcomere lengths \( \geq 2.2 \mu m \), during which cells in the center shorten only 2% to 3% at the expense of damaged ends (23), is analogous to an unphysiologically severe pressure overload; the resulting decrease in \( L/D \) ratio is even larger than that typically seen in experimental pressure overload. By our hypothesis, cultured papillary muscles experiencing physiological amounts and rates of shortening should maintain a normal \( L/D \) ratio, as observed in this study (Fig. 4). We note that although the term “shortening” is a convenient shorthand, since each contraction is followed by diastolic stretching of the same magnitude and the rates of both were fixed in our experiments, we cannot yet comment on the relative importance of the amounts and rates of systolic shortening and diastolic lengthening as potential governing stimuli for myocyte shape.

**Potential contributions of sarcomere rearrangement.** Three different observations each suggest that part of the myocyte shape change we observed in isometrically cultured muscles was due to the rearrangement of existing sarcomeric proteins rather than synthesis of new protein, but none of these observations is conclusive. First, we did not observe a change in myocyte volume during isometric culture; however, the statistical power of this test was very low. Second, the 27% increase in myocyte diameter we observed after 36 h of culture translates to a rate of increase in myocyte CSA of 41%/day, nearly twice the rate of in vivo protein synthesis measured by Moalic et al. (17) in rat myocardium during pressure-overload hypertrophy (25%/day). Finally, culture of muscles in the presence of 100 \( \mu M \) puromycin had no effect on myocyte CSA increases; however, Mansour et al. (16) found that 24 h of pretreatment with puromycin were required to block stretch-induced sarcomere remodeling in cultured myocytes, and we did not pretreat animals with puromycin before muscle harvest. Taken together, these observations suggest, but do not prove, that rearrangement of sarcomeric proteins may explain part of the myocyte shape change we observed in isometrically cultured muscles.

**Limitations and sources of error.** Critical potential errors and artifacts were excluded by appropriate control experiments as discussed in results. In particular, any artifacts of the culture system itself should have exerted their effects in all muscles, so maintenance of cell size and shape in shortening muscles was convincing evidence against a prominent role for such artifacts. In muscles cultured isometrically for 36 h, there was no evidence of intracellular edema on blinded electron microscopy analysis and no change in the normal relationship between myocyte CSA and distance from the papillary muscle surface. This last point is important because myocytes in cultured muscles rely on diffusion from the surface to supply oxygen and other nutrients. If the myocyte size and shape changes we observed in isometrically cultured papillary muscles had been due largely to hypoxia or to the availability of glucose, amino acids, or other nutrients, there should have been a shift in the slope of the relationship between myocyte CSA and location, with CSA increases occurring preferentially either near the center or near the periphery of the muscles. However, the interpretation of data from any in vitro experimental system merits caution. Right ventricular papillary muscles and trabeculae may differ from the bulk of the right and left ventricular myocardium in ways that could affect the interpretation of our results. The fiber structure in papillary muscles and trabeculae certainly differs from that of the wall, with fibers aligned uniaxially along the long axis of the preparation rather than arrayed in a more complex three-dimensional pattern. In addition, possible differences in mechanical history between trabeculae or papillary muscles and myocardium in the heart wall could affect interpretation of mechanical perturbations in culture since the in vivo mechanical state forms the baseline for these perturbations. However, measurements of papillary muscle mechanics in situ show shortening patterns very similar to midwall circumferential shortening (20), and both papillary muscles (1) and trabeculae (9) have been shown to hypertrophy during in vivo hemodynamic overload.

**Conclusions.** The hypothesis that elevated systolic stress induces myocyte thickening has been difficult to test directly. We tested this hypothesis in working rat right ventricular papillary muscles using a recently developed technique for long-term muscle culture (12). Isometric contraction induced rapid increases in myocyte diameter regardless of the level of systolic stress, whereas control myocyte dimensions were maintained if physiological amounts and rates of systolic shortening were imposed. Myocyte thickening was accompanied by a decrease in cell length and the number of sarcomeres in series along the long axis of the myocyte, suggesting that thickening may have occurred in part by rearrangement of existing sarcomeres. We conclude that the pattern of systolic shortening and/or diastolic lengthening regulates myocyte shape in working rat right ventricular papillary muscles, whereas systolic stress plays little or no role.

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