LOAD- AND TIME- DEPENDENT MYOCYTE SIZE INCREASES IN ISOMETRICALLY CONTRACTING CARDIAC MUSCLE

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INTRODUCTION

Increased mechanical load is a proposed stimulus for hypertrophic growth of adult myocardium [1-5]. Mechanical stimuli may operate by exerting an increased mechanical stress and/or strain on the myocardium [1-5]. Myocytes respond to in vivo pressure overload with parallel addition of sarcomeres, resulting in increased myocyte cross-sectional area and ventricular wall thickness [1]. However, determining the precise role of mechanical stimuli in the in vivo response of hypertrophic growth is difficult [4].

Previous studies have exploited animal models, intact myocardium, and cultured cells to study the effect of increased mechanical load on the growth of cardiac myocytes [2-5]. The use of intact contracting myocardium allows for precise control and measurement of increased active and passive load, and thus insight into the precise role of mechanical stress and strain on growth. In addition, intact myocardium possesses potentially important signaling pathways for growth such as an extracellular matrix with cell-to-cell interactions as well as cellular heterogeneity with potential autocrine and paracrine interactions. Thus, this study utilizes a novel apparatus, the trabecula culture system [6], to maintain contraction of intact myocardium for multiple days in an effort to study growth due to known increases in mechanical load.

MATERIALS AND METHODS

Experimental Apparatus. Contraction of right ventricular papillary muscles for up to 76 hours was maintained using the trabecula culture system [6]. Briefly, the system consists of an airtight muscle chamber through which 95%O_2-5%CO_2 is pumped. The muscle bathes in a solution volume of ~3 mL while a stir bar continuously circulates the solution producing a pO_2 greater than 450 mmHg. The solution was held at a constant temperature of 37.0 +/-0.2 ^oC and pH of 7.35 - 7.45. Bacterial and/or fungal contamination was prevented by sterilizing all parts of the system that would come in contact with the solution or muscle chamber.

Muscle Isolation. Male LBN-F1 rats, 250–350 g, (Harlan Sprague Dawley) were anesthetized by intraperitoneal injection of 94 mg/kg pentobarbital. After intracardiac heparinization, the hearts were rapidly dissected and perfused through the aorta with a Krebs-Henseleit solution containing (in mM) 118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 20 $NaHCO_3$, 11 glucose, 0.25 $CaCl_2$, 30 2,3-butanedione monoxime (BDM), and 20 IU/L insulin in equilibrium with 95% O_2–5% CO_2. Two right ventricular papillary muscles were dissected from each rat.

Experimental Protocol. The following experimental protocol was applied simultaneously to two muscles obtained from a single rat. Muscles were mounted in the trabecula culture system between a hook extension of a force transducer and a hook connected to a micromanipulator. The solution was exchanged for a BDM-free Krebs-Henseleit solution. Muscles were stimulated end-to-end at ~30% above threshold voltage (2-4 V) with 0.5 ms asymmetric pulses, at a frequency of 0.5 Hz. The calcium concentration of the Krebs-Henseleit solution was then raised stepwise to 1.75 mM.

With the use of the micromanipulator, each muscle was stretched until additional stretch did not produce an increase in developed contractile stress. This length, L_{max}, unique for each muscle, represents a sarcomere length of ~2.4μm [7]. After a brief period of equilibration, each muscle was shortened to 80% L_{max} or 95% L_{max}. After force stabilization, the Krebs-Henseleit solution was exchanged for a modified cell culture medium (M199, Sigma) with additions (in mM): 2.0 L-carnitine, 5.0 creatine, 5.0 taurine, 2.0 L-glutamine, 100 IU/mL penicillin, 0.1 mg/mL streptomycin, 20 IU/l insulin. Active systolic and passive diastolic forces were acquired using a custom-designed data acquisition program (LabView, NI). Both medium and gas were changed at regular intervals of 7-11 h under sterile conditions. Termination of contraction (pre-determined or due to a low magnitude of developed force) was done with the addition of a Krebs-Henseleit solution containing 30 mM BDM. In some cases, contraction terminated spontaneously before the addition of BDM. Muscle dimensions were determined at 80% or 95% L_{max} using a 40X microscope.

Histology. Muscles were fixed at rest length in 10% formalin. After dehydration and infiltration, muscles were embedded in glycol

methacrylate (EBSciences). Muscle short axis sections (6μm) were stained with Movat's Silver Impregnation and imaged at 200X. Cell outlines were manually traced using Scion Image (NIH) to determine the average cell cross-sectional area for each muscle (100-400 cells/muscle) according to the criteria set by Omens et al [2].

RESULTS

A total of twelve right ventricular papillary muscles were studied (80% L_{max}: n=8; 95% L_{max}: n=4). Muscle length averaged 2.53 ± 0.88 mm and radius 0.24 ± 0.06 mm. At L_{max}, the average developed contractile stress (F_{dev}) was 14.1 ± 6.0 mN/mm^2.

Mechanical Parameters. Both average developed contractile stress and passive diastolic stress (F_{diast}) were higher at longer muscle lengths. During the first 24 hours, F_{dev} in muscles that contracted at 95% L_{max} was 212% larger then F_{dev} in muscles that contracted at 80% L_{max} (7.8 ± 4.9 vs. 2.5 ± 1.3 mN/mm^2, P < 0.03). Passive diastolic stress (F_{diast}) was 1.3 ± 0.9 mN/mm^2 and 8.9 ± 1.6 mN/mm^2 in muscles that contracted at 80% L_{max} and 95% L_{max}, respectively (P < 0.01). In muscles that contracted beyond 24 hours (n = 9) the magnitude of F_{dev} and F_{diast} during the first 24 hours was compared to magnitudes after 24 hours (time varied from muscle to muscle). A paired t-test indicated that F_{dev} decreased significantly (P < 0.01) and F_{diast} increased significantly (P < 0.03), indicating gradual decay of the preparation beyond 24 h.

Initial Myocyte Area. In Figure 1A, t= 0 h represents muscles that did not contract in the muscle bath (n=6). Average myocyte area was 273 ± 9 μm^2 and was significantly less then myocyte area of muscles that contracted at 80% L_{max} for t < 36 h (P < 0.01).

Contraction Time vs. Myocyte Area. In Figure 1A, average myocyte area from muscles that contracted at 80% and 95% L_{max} for less than 36 h (t < 36 h, n = 4), between 36 and 60 h (t = 36–60 h, n = 5), and for longer than 60 h (t > 60 h, n = 3) is shown. A significant increase in myocyte area with contraction time was found (ANOVA P = 0.0001).

Muscle Length vs. Myocyte Area. In Figure 1B, myocyte cross-sectional area from paired muscles that contracted at 80% L_{max} and 95% L_{max} is shown. Each time point represents two muscles taken from a single rat. Myocyte area was significantly larger in muscles that contracted at 95% L_{max} compared to 80% L_{max} (P < 0.002). In these muscles, an increase in contractile length from 80% to 95% L_{max} produced, on average, a 13% increase in myocyte area.

DISCUSSION

The results of this study indicate that isometric contraction of isolated intact cardiac muscle is associated with an increase in myocyte cross-sectional area over time. The magnitude of active and passive load was greater, and the increase in myocyte area was faster at longer relative muscle lengths.

From this data, the rate of increase in myocyte area, indicative of growth rate, was found to be 47% and 35% per day for muscles contracting at 95% and 80% L_{max}. Omens et al. [2] found rat left ventricular free wall myocytes increased area at a rate of 12% per day in a pressure overload animal model. Invester et al. [4] found increased protein synthesis rates of 43% after 4 h of electrical stimulation in cultured adult feline cardiocytes. Decker et al. [3] reported a growth rate of 8% and 2 % per day in electrically paced and passively stretched adult feline cardiocytes. Thus, growth rates found in this study using intact contracting cardiac muscle are larger than those reported previously in animal models and cultured cells [2,3].

To conclude, an increase in myocyte area, dependent on both contraction time and relative muscle length, was found. This data suggests that the increased mechanical load at longer muscle lengths may be responsible for the increase in myocyte area. In addition, several other factors may contribute to the increase in area including edema or the increased surface area of muscle available for transport of growth factors at longer muscle lengths. Future studies will explore these possibilities.

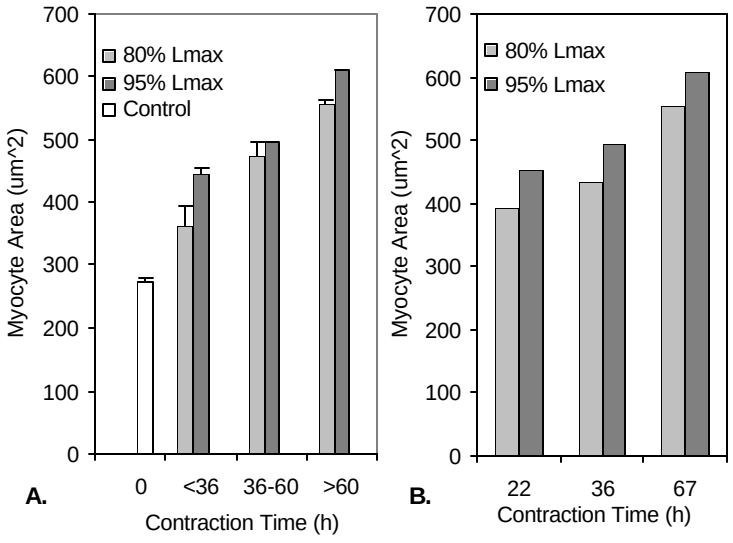

Figure 1.

A. Myocyte area of actively loaded intact cardiac muscle increases with load time (ANOVA P=0.0001).

B. Myocyte area of paired actively loaded intact cardiac muscle at 95% vs. 80% L_{max} (P < 0.002).

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REFERENCES

1. Grossman, W., Jones, D., McLaurin, L.P., 1975, "Wall stress and patterns of hypertrophy in the human left ventricle," J Clin Invest, 56, pp56-64.
2. Omens, J.H., Rodriguez E.K., McCulloch, A.D. 1996, "Transmural changes in stress-free myocyte morphology during pressure overload hypertrophy in the rat," J Mol Cell Cardiol, 28, pp1975-1983.
3. Decker, M.L., Janes, D.M., Barclay, M.M., Harger, L., Decker, R.S. 1997, "Regulation of adult cardiocyte growth: effects of active and passive mechanical loading," Am J Physiol., 272, H2902-H2918.
4. Invester, C.T., Kent, R.L., Tagawa, H., Tsutsui, H., Imamura, T., Cooper, G., McDermott, P.J. 1993, "Electrically stimulated contraction accelerates protein synthesis rates in adult feline cardiocytes," Am J Physiol., 265, H666-H674.
5. Peterson, M.B., Lesch, M. 1972, "Protein synthesis and amino acid transport in the isolated rabbit right ventricular papillary muscle," Circ Res., 31, pp 317-327.
6. Janssen, P. M., Lehnart, S. E., Prestle, J., Lynker, J.C., Salfeld, P., Just, H., and Hasenfuss, G. 1998, "The trabecula culture system: a novel technique to study contractile parameters over a multiday time period," Am J Physiol, 274, H1481-H1488.
7. Rodriguez, E.K., Hunter, W.C., Royce, M.J., Leppo, M.K., Douglas, A.S., Weisman, H.F. 1992, "A method to reconstruct myocardial sarcomere lengths and orientations at transmural sites in beating canine hearts," Am. J. Physiol., 263, H293-H306.