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Myocardial relaxation is accelerated by fast stretch, not reduced afterload

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ABSTRACT

Fast relaxation of cross-bridge generated force in the myocardium facilitates efficient diastolic function. Recently published research studying mechanisms that modulate the relaxation rate has focused on molecular factors. Mechanical factors have received less attention since the 1980s when seminal work established the theory that reducing afterload accelerates the relaxation rate. Clinical trials using afterload reducing drugs, partially based on this theory, have thus far failed to improve outcomes for patients with diastolic dysfunction. Therefore, we reevaluated the protocols that suggest reducing afterload accelerates the relaxation rate and identified that myocardial relengthening was a potential confounding factor. We hypothesized that the speed of myocardial relengthening at end systole (end systolic strain rate), and not afterload, modulates relaxation rate and tested this hypothesis using electrically-stimulated trabeculae from mice, rats, and humans. We used load-clamp techniques to vary afterload and end systolic strain rate independently. Our data show that the rate of relaxation increases monotonically with end systolic strain rate but is not altered by afterload. Computer simulations mimic this behavior and suggest that fast relengthening quickens relaxation by accelerating the detachment of cross-bridges. The relationship between relaxation rate and strain rate is novel and upends the prevailing theory that afterload modifies relaxation. In conclusion, myocardial relaxation is mechanically modified by the rate of stretch at end systole. The rate of myocardial relengthening at end systole may be a new diagnostic indicator or target for treatment of diastolic dysfunction.

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1. Introduction

Rapid relaxation after ejection is essential for efficient filling of the left ventricle of the heart [1]. If the heart relaxes too slowly, there is not enough time for the heart to fill and cardiac function is compromised. Clinically, slow relaxation is an independent diagnostic criterion for Heart Failure with preserved Ejection Fraction (HFpEF) [2]. Improved understanding of the mechanisms that influence the rate of relaxation could lead to better treatments for diastolic dysfunction and HFpEF.

Cardiac relaxation ultimately reflects the reduction in the number of myosin heads that are bound to actin. This is a very complex process, and several molecular-level mechanisms are known to influence the rate of relaxation [3]. For example, myocardial relaxation can be quickened by increasing SERCA expression [4] or slowed by decreasing SERCA activity [5]. Increasing SERCA expression reduces the intracellular Ca^{2+} concentration during the latter stages of a twitch contraction, which in turn decreases the number of binding sites on actin to which myosin heads can attach.

Mechanical factors that influence relaxation have received less attention since the 1980s. Seminal work by D. L. Brutsaert and others used isolated intact trabeculae and working hearts to establish the theory that reducing afterload accelerates the myocardial relaxation rate [6–9]. In part due to the high prevalence of hypertension in patients with Heart Failure with preserved Ejection Fraction, clinical trials attempted to treat such patients with anti-hypertensive therapies such as inhibition of the renin-angiotensin-aldosterone system [10]. However, reducing afterload did not improve outcomes in these patients.

We were intrigued by these results and recent reports of reduced myocardial strain rates in HFpEF patients [11]. Therefore, we re-evaluated the original protocols used to suggest that afterload modifies the relaxation rate [6,7,9,10]. We noted that both afterload and the myocardial strain rate at end systole (the speed of muscle relengthening) are simultaneously adjusted when using the conditions of the original protocol. However, the simultaneous change in afterload and strain rate suggests that strain rate may have been a confounding factor in the interpretation of the seminal experiments. The authors

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concluded that afterload was the mechanical modifier of relaxation, even though they noted that relengthening is necessary to modify relaxation [6,9,12].

This manuscript describes new experiments that we developed to isolate the role of myocardial strain rate from afterload. Specifically, we independently modified afterload and end-systolic strain-rate in experiments using electrically simulated trabeculae from mice, rats, and humans. We also used computer simulations to investigate the relationship between fast stretch and cross-bridge detachment. Our data shows a novel relationship between relaxation rate and end systolic strain rate and the likely molecular mechanism is enhanced cross-bridge detachment.

2. Methods

2.1. Isolation of rodent ventricular trabecula

Female Sprague-Dawley or wild-type (mixed Sprague-Dawley/F344/Brown Norway) rats [13] and male C57BL/6 mice were used in this study. Animal use was approved by the Institutional Animal Use and Care Committees of the University of Kentucky and Wayne State University.

Each animal was anesthetized via an IP injection of sodium pentobarbital (50 mg kg^{-1}) or inhalation of 3% isoflurane and heparinized via an IP administration of sodium heparin (1000 U kg^{-1}). The animal was subsequently euthanized by exsanguination, and its heart was rapidly excised and rinsed in a cold (4 °C), oxygenated (>10 ppm) perfusion solution (in mM: 113 NaCl, 4.7 KCl, 0.6 KH_2PO_4, 1.2 $MgSO_4$, 12 $NaHCO_3$, 10 $KHCO_3$, 10 2-[4-2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), 30 Taurine, 5.5 glucose, 10 2,3-butanedione monoxine (BDM)). Additional perfusion solution was then flushed through the heart via an aortic cannula to remove any remaining blood. The heart was then placed in a dissecting dish that was coated with Sylgard (Dow Corning, Midland MI) and filled with cold perfusion solution. After opening the ventricles, the endocardial surfaces were inspected for free-standing trabeculae, including cylindrical papillary muscles. Potential preparations were removed from the heart by cutting a cuboid section of ventricular wall at each end of the trabecula. Contact with the trabecula itself was minimized to reduce the probability of induced damage.

2.2. Isolation of human ventricular trabecula

One set of experiments was performed using a trabecula isolated from the right ventricle, between the mid-wall and apex, of an organ donor (60 year old female). The organ was not transplantable due to the donor's history of left coronary artery disease. This experiment was approved by the Institutional Review Board of the University of Kentucky.

During the organ procurement, the heart was perfused with cardioplegia solution. The ventricles were subsequently excised and provided to the research team submerged in cardioplegia solution. A free-standing trabecula was located on the endocardial surface of the right ventricular free wall and carefully isolated from the organ.

2.3. Intact trabecula mechanics

Each trabecula was transferred from the dissecting dish to the experimental chamber inside a bulb pipette using perfusion solution to maintain hydration. This prevented the trabecula from dehydrating during the transfer procedure. The experimental chamber had a volume of 350 μL and was continuously perfused with oxygenated Tyrode's Solution (in mM: 140 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 glucose) at 25 °C or in one case increased to 37 °C. One end of the trabecula was hooked to a high-speed length motor (Model 312C or 315C, Aurora Scientific, Aurora, Ontario, Canada) while the other end was secured to a force transducer (Model 403, Aurora Scientific). The trabecula was paced at 0.5 Hz using bipolar excitation approximately 1.2 times the threshold voltage and stretched to L_0, the length where the maximal force was developed during isometric contractions. L_0 and the cross sectional area were measured by video microscopy. The cross-sectional area was calculated by averaging the diameter of the trabecula at 4 positions along its length and assuming a cylindrical geometry. It was allowed to equilibrate for approximately 1 h before data acquisition began.

Experiments were performed using SLControl software [14] and a newly written real-time control algorithm. The trabecula was held isometric except for the experimental load-clamped twitches that were performed at least 4 s apart to prevent history-dependent effects [15]. During a load-clamp trial, the trabecula isometrically contracted until force reached a predefined set-point (the afterload). Force was then isotonically maintained at the chosen afterload by adjusting the motor position to shorten and then relengthen the muscle in real-time using the control algorithm. If the load-clamp was stopped and the muscle was held isometric before the minimum length was achieved, additional systolic force was generated before the muscle could relax (Supplemental Fig. S1). To focus on the mechanical behaviors of relaxation, the load-clamp was terminated and the muscle allowed to relax isometrically after one of three events. These events were: 1) the trabecula relengthened back to its original length, 2) the trabecula began to relengthen (i.e, the trabecula was held at the minimum attained length), or 3) the trabecula relengthened by a pre-set amount. Different termination criteria were used in successive trials to investigate different mechanical behaviors. Once the load-clamp was terminated, the trabecula was allowed to relax under isometric conditions at that length. If necessary, the trabecula was returned to its original length after the trial was complete. Load-clamps using different termination conditions were repeated at various afterloads (typically between 25 and 75% of the maximum isotonic force). Experiments were stopped if the developed isometric force decreased by >20% from the equilibrated maximal force at L_0, which typically occurred >6 h after the muscle was equilibrated.

Data were analyzed offline using custom scripts written in MATLAB (The MathWorks, Natick, MA). Force records were smoothed using a Savitzky-Golay filter and normalized to the measured cross-sectional area. Data were aligned using either the stimulus pulse or a threshold force when the stimulus was not available for technical reasons. Changes in muscle strain were calculated from the muscle length and the position of the motor. No corrections were made to allow for series compliance in the attachments to the experimental apparatus. For load-clamp twitches, the onset of relaxation was defined as the time-point at which the motor stopped moving. The relaxation rate was determined using the Glantz Method calculated in the pressure-phase plane [16,17]. The rate is defined as 1/tau, where tau is the typical time constant of isovolumic relaxation.

2.4. Mathematical modeling

Twitch contractions were simulated using a mathematical model consisting of a single half-sarcomere connected in series to an elastic spring. The half-sarcomere was represented by a population of cross-bridges cycling through a 2-state scheme and a parallel elastic component that produced the system's passive resting tension. Strain dependent myosin kinetics were included in the model. The series elastic spring was non-linear and mimicked the compliance in the trabecula's attachments to the experimental apparatus. All calculations were performed using freely-available MyoSim software [18] that can be downloaded from http://www.myosim.org. The data files necessary to reproduce these simulations are included as Supplementary material.

The half-sarcomere was activated in every simulation by an identical idealized Ca^{2+} transient of the form

$$Ca(t) = \begin{cases} Ca_{diastolic} & t \le t_{stimulus} \\ \left(\frac{Ca_{amplitude} - Ca_{diastolic}}{\beta}\right)\left(e^{-\frac{t-t_{stimulus}}{\tau_1}} - e^{-\frac{t-t_{stimulus}}{\tau_2}}\right) + Ca_{diastolic} & t > t_{stimulus.} \end{cases}$$

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