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Increased cross-bridge recruitment contributes to transient increase in force generation beyond maximal capacity in human myocardium

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ABSTRACT

Cross-bridge attachment allows force generation to occur, and rate of tension redevelopment (k_{tr}) is a commonly used index of cross-bridge cycling rate. Tension overshoots have been observed briefly after a slack-restretch k_{tr} maneuver in various species of animal models and humans. In this study, we set out to determine the properties of these overshoots and their possible underlying mechanism. Utilizing human cardiac trabeculae, we have found that tension overshoots are temperature-dependent and that they do not occur at resting states. In addition, we have found that myosin cross-bridge cycle is vital to these overshoots as inhibition of the cycle results in the blunting of the overshoots and the magnitude of the overshoots are dependent on the level of myofilament activation. Lastly, we show that the number of cross-bridges transiently increase during tension overshoots. These findings lead us to conclude that tension overshoots are likely due to a transient enhancement of the recruitment of myosin heads into the cross-bridge cycling, regulated by the myocardium, and with potential physiological significance in determining cardiac output.

News and noteworthy: We show that isolated human myocardium is capable of transiently increasing its maximal force generation capability by increasing cross-bridge recruitment following slack-restretch maneuver. This process can potentially have important implications and significance in cardiac contraction in vivo.

1. Introduction

Cardiac stroke volume is determined by several factors, most notably the amount of pressure the heart can generate during each cardiac cycle [1]. One of the most important factors determining this pressure is myocardial contractility [1]. Increasing calcium concentration results in increased thin filament activation and enhanced cross-bridge recruitment which ultimately leads to increased tension generation [2]. This relationship reaches a maximal steady state where a further increase in calcium concentration has no effect on tension. At this point, the number of attached cross-bridges is at a relative steady state, and cardiomyocytes have reached their maximal tension development capacity [3].

Rate of tension redevelopment (k_{tr}), a kinetic parameter commonly used as an index of cross-bridge cycling rate, utilizes a rapid slack-restretch maneuver [4]. The tension that cardiomyocytes generate following the rapid slack-restretch maneuver can briefly exceed the tension developed during maximal myofilament activation preceding the maneuver. Such tension "overshoots" have been reported in intact human cardiac trabeculae [5,6], permeabilized human cardiac preparations [7], permeabilized mouse cardiac preparations [8], intact rat cardiac trabeculae, and permeabilized rat cardiomyocytes upon Protein Kinase A (PKA) treatment [9] and unpublished experiments in permeabilized porcine, canine, and rat cardiac preparations [10]. Our laboratory has been working with intact human ventricular trabeculae and have observed tension overshoots in vast majority of the non-failing as well as failing trabeculae with similar amplitudes of overshoot.

It is incompletely understood whether tension overshoots are simply a passive experimental artifact or they occur due to an active intrinsic mechanism of the myocardium itself. We hypothesized that tension overshoots are an active physiological intrinsic property. In the present

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Table 1
Characteristics of human hearts.

Heart	Type	Age	Gender	Race	Temperature	Resting	BDM	Sub-Maximal	Sinusoidal
442,404[a]	Donor	69	Male	Caucasian	X				
118,258[a]	Donor	38	Male	Caucasian	X				
768,159[a]	Donor	44	Male	African-American	X		X		
600,245[a]	Donor	51	Female	Caucasian	X				
156,910	Donor	62	Female	Caucasian		X			
600,245	Donor	51	Female	Caucasian			X		
685,884[a]	Donor	36	Male	Caucasian				X	
474,083[a]	Donor	41	Female	African-American				X	
481,043[a]	Donor	65	Female	Caucasian				X	
947,200[a]	Donor	63	Female	Caucasian				X	
476,074[a]	Donor	29	Female	Caucasian		X		X	
240,603[a]	Donor	51	Female	Caucasian		X		X	
452,192[a]	Donor	55	Female	African-American				X	
987,692	Donor	34	Female	Caucasian					X
479,062[a]	Failing	50	Male	Caucasian				X	
323,104[a]	Failing	63	Male	Caucasian				X	
537,263[a]	Failing	62	Male	African-American		X		X	
522,421[a]	Failing	56	Female	Caucasian				X	
214,010[a]	Failing	64	Male	Caucasian				X	
597,750[a]	Failing	67	Male	Caucasian		X		X	
806,554	Failing	39	Female	African-American		X			
378,549	Failing	47	Male	Caucasian					X
554,147	Failing	56	Male	Caucasian					X
897,154	Failing	53	Female	African-American					X
437,918	Failing	40	Male	Caucasian					X
187,899	Failing	32	Female	Caucasian			X		
388,426	Failing	58	Female	Caucasian	X		X		

[a] Indicate hearts reported in previous studies [5,6].

study, we show that tension overshoots are impacted by factors such as temperature, myofilament activation level, electrical activation of myocytes, and myosin activity. Finally, we show that a transient increase in cross-bridge recruitment is the underlying mechanism for transient tension overshoots suggesting an additional mechanism whereby the myocardium can enhance its force generation capacity.

2. Materials and methods

2.1. Human heart procurement

All experiments were performed with approval of the Institutional Review Board (IRB) at The Ohio State University Wexner Medical Center and The Declaration of Helsinki. Donor non-failing human hearts (n = 14) were acquired in collaboration with the LifeLine of Ohio Organ Procurement. Failing hearts (n = 13) were acquired with informed consent from patients undergoing cardiac transplantation at The Ohio State University Wexner Medical Center. Table 1 provides characteristics of the human hearts which include a subset of hearts that have been reported in our previous studies [5,6]. Following explantation, human hearts were immediately flushed with a cold cardioplegic solution containing (in mM): 110 NaCl, 16 KCl, 16 $MgCl_2$, 10 $NaHCO_3$, and 0.5 $CaCl_2$ and transported promptly to the laboratory in this solution.

2.2. Isolation of intact cardiac trabeculae

The right ventricles were dissected from the remainder of the hearts and transferred to a cold (0–4 °C) modified Krebs-Henseleit solution (K−H) containing (in mM): 137 NaCl, 5 KCl, 20 $NaHCO_3$, 1.2 NaH_2PO_4, 1.2 $MgSO_4$, 10 dextrose, 0.25 $CaCl_2$,and 20 BDM (2,3-butanedione monoxime). The solution was in equilibrium with 95% O_2–5% CO_2 resulting in pH of 7.4. Right ventricular cardiac trabeculae were isolated and stored in K−H solution at 0–4 °C until the time of the experiment.

Trabeculae were placed in a custom-made setup to a force transducer and a linear motor via two pairs of hooks on each side as previously described [5,11]. Preparations were continuously superfused with a modified K−H solution without BDM at 37 °C. Trabeculae were stimulated to contract at 1 Hz and the $CaCl_2$ concentration was incrementally and gradually increased to 2 mM. Afterwards, trabeculae were stretched to optimal length, close to in vivo end-diastolic sarcomere length of 2.2 μm [12], as previously described. The K^+ contractures and k_{tr} experimental protocol were performed as previously detailed [3,5]. All experiments, unless otherwise specified, were performed at optimal length, 1 Hz, and 37 °C.

2.3. Temperature dependency of tension overshoots

Four trabeculae (n = 4 donor hearts, n = 1 failing heart) were used to assess the amplitude and the decline phase of the tension overshoots at different temperatures. K_{tr} experiments were performed at optimal length, 1 Hz, and temperatures of 37 °C, 32 °C, and 27 °C in each of the muscles.

2.4. Tension overshoots in resting conditions

Four trabeculae (n = 2 donor hearts and n = 2 failing hearts) were used to determine whether tension overshoots occur in non-contracting trabeculae. The electrical stimulation was paused and 3–4 successive k_{tr} maneuvers were performed without inducing a K^+ contracture.

Two additional trabeculae were initially stabilized at L_{opt} and 1 Hz for 15 min. K^+ contractures were induced and the k_{tr} maneuvers were performed at maximal contracture tension. Afterwards, the trabecula was re-stabilized at L_{opt} and 1 Hz for 15 min. The electrical stimulation was paused and the trabeculae were stretched until the resting tension was approximately equal to the total tension of the preceding K^+ contracture. A k_{tr} maneuver was performed at this high resting tension level. The trabecular length was returned to L_{opt} and re-stabilized at 1 Hz for 15 min. Subsequently, K^+ contracture and k_{tr}, at maximal contracture tension, were performed.