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Insights into length-dependent regulation of cardiac cross-bridge cycling kinetics in human myocardium

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ABSTRACT

Cross-bridge cycling kinetics play an essential role in the heart's ability to contract and relax. The rate of tension redevelopment (k_{tr}) slows down as a muscle length is increased in intact human myocardium. We set out to determine the effect of rapid length step changes and protein kinase A (PKA) and protein kinase C-βII (PKC-βII) inhibitors on the k_{tr} in ultra-thin non-failing and failing human right ventricular trabeculae. After stabilizing the muscle either at L_{90} (90% of optimal length) or at L_{opt} (optimal length), we rapidly changed the length to either L_{opt} or L_{90} and measured k_{tr}. We report that length-dependent changes in k_{tr} occur very rapidly (in the order of seconds or faster) in both non-failing and failing muscles and that the length at which a muscle had been stabilized prior to the length change does not significantly affect k_{tr}. In addition, at L_{90} and at L_{opt}, PKA and PKC-βII inhibitors did not significantly change k_{tr}. Our results reveal that length-dependent regulation of cross-bridge cycling kinetics predominantly occurs rapidly and involves the intrinsic properties of the myofilament rather than post-translational modifications that are known to occur in the cardiac muscle as a result of a change in muscle/sarcomere length.

1. Introduction

The beating heart utilizes the Frank–Starling mechanism to increase its cardiac output as its volume increases due to venous return of blood [1]. As the diastolic ventricular volume increases, not only does the contractile force increase, but the kinetics of contraction and relaxation are also modulated [2,3]. Cross-bridge cycling kinetics is an important contributor in determining cardiac output [4–6] and consequently discovering how this kinetic parameter is regulated is essential to having a comprehensive understanding of how the heart regulates its pumping activity, is altered in heart failure, and discovering novel potential therapeutic targets. On a cellular level, it has been shown that increasing muscle length, an *in vitro* index of pre-load, decreases cross-bridge cycling kinetics in animal models [7–11]. We have recently shown that this length-dependent regulation of cross-bridge cycling kinetics is also present in both non-failing and failing human myocardium under conditions close to those *in vivo* [12]. The exact mechanism(s) by which muscle length affects cross-bridge cycling kinetics remains however unknown.

It has been proposed that stretching cardiomyocytes results in stretching of titin which interacts with cardiac myosin binding protein-C (cMyBP-C). This in turn exerts movement restriction on myosin heavy chain (MHC) that ultimately results in decreased cross-bridge cycling rate [5,10,13]. Another possible mechanism is that increasing muscle length induces post-translational modifications of contractile proteins and these modifications are responsible for the length-dependent regulation of contractile kinetics. It is well known that modifications including

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phosphorylation of contractile proteins, such as myosin light chain 2 (MLC-2), cMyBP-C and Troponin-I (TnI) Ser23/24, are important determinants of cross-bridge cycling kinetics [14–22]. Recent studies have shown that increasing pre-load is associated with alterations in MLC-2 phosphorylation of rat myocardium [23]. Furthermore, increasing muscle length has been shown to alter phosphorylation of MLC-2 in human [24] and both MLC-2 and TnI Ser23/24 in rabbit cardiac muscles [3], while stretching permeabilized guinea pig ventricular cardiomyocytes is associated with increased phosphorylation of MLC-2 and cMyBP-C [25]. Furthermore, we have previously shown that Protein Kinase A (PKA) and Protein Kinase C-βII (PKC-βII) pathways are essential to this length-dependent phosphorylation and their inhibition results in faster twitch kinetics at increased muscle lengths [26]. Thus, length-dependent phosphorylation of contractile proteins is a plausible explanation for the effects of muscle length on cross-bridge cycling kinetics which warrants further evaluation.

We previously developed a novel method for assessing cross-bridge cycling kinetics by measuring rate of tension redevelopment (k_{tr}) in intact cardiac trabeculae [7]. This technique has the advantage of conducting experiments on intact cardiac preparations under near-physiological conditions, where the post-translational modification machinery remains intact. It allows the possibility of assessing whether the length-dependent regulation of contractile kinetics is solely dependent on the inherit properties of the myofilaments or that post-translational modifications also have a main role. In this study, we show that length-dependent regulation of cross-bridge cycling kinetics is a near instantaneous process suggesting that the underlying mechanism is within the myofilament proteins.

2. Methods

2.1. Procurement of human hearts

All experiments on human tissue presented in this study were performed in accordance with the Institutional Review Board (IRB) at The Ohio State University and Declaration of Helsinki. Non-failing human hearts not suitable for cardiac transplantation (n = 9) were acquired from LifeLine of Ohio Organ Procurement and failing hearts (n = 8) from patients undergoing cardiac transplantation at The Ohio State University Wexner Medical Center. Informed consent was acquired from all patients undergoing cardiac transplantation. Human hearts reported here are a subset of samples that were used in our previous study [12]. The details of the human hearts and type of experiment performed are outlined in Table 1. The acquired hearts were immediately flushed with cold cardioplegic solution containing (in mM): 110 NaCl, 16 KCl, 10 $NaHCO_3$, 16 $MgCl_2$, and 0.5 $CaCl_2$ and transported promptly to the laboratory.

2.2. Isolation of cardiac trabeculae

The right ventricles were transferred from the cardioplegic solution to a Krebs–Henseleit solution (K–H) previously bubbled with 95% O_2–5% CO_2 containing (in mM): 137 NaCl, 5 KCl, 20 $NaHCO_3$, 10 dextrose, 1.2 NaH_2PO_4, 1.2 $MgSO_4$, 0.25 $CaCl_2$, and 20 BDM (2,3-butanedione monoxime) and pH of 7.4. Small linear trabeculae were isolated from the right ventricles. All trabeculae were submerged in this K–H solution and kept at 0–4 °C until the start of the experiments. Muscles were mounted on a custom-made setup as previously described [7,12]. The perfusion solution was a modified K–H solution (37 °C) without BDM and containing an initial $CaCl_2$ concentration of 0.25 mM. Muscles were stimulated at 1 Hz and the $CaCl_2$ concentration was gradually raised to 2 mM. The muscles were then gradually stretched to optimal length (L_{opt}) as previously described [12] which is close to the sarcomere length of 2.2 μm at end-diastole *in vivo* [27].

Table 1
Characteristics of human hearts.

Heart	Age	Gender	Race	Inhibitors	Rapid length change
Non-Failing					
685884	36	Male	Caucasian	–	X
474083	41	Female	African-American	–	X
618200	58	Female	Caucasian	X	X
481043	65	Female	Caucasian	–	X
947200	63	Female	Caucasian	X	X
476074	29	Female	Caucasian	X	–
240603	51	Female	Caucasian	X	X
452192	55	Female	African-American	X	–
118258	38	Male	Caucasian	X	–
Failing					
450564	30	Male	Caucasian	–	X
328163	63	Male	Caucasian	–	X
479062	50	Male	Caucasian	–	X
323104	63	Male	Caucasian	X	X
537263	62	Male	African-American	X	X
522421	56	Female	Caucasian	X	X
214010	64	Male	Caucasian	X	X
597750	67	Male	Caucasian	X	X

Most heart are subset of hearts used in a previous study [12]. X indicates that the heart was used for the experiment.

2.3. k_{tr} measurements with inhibitors

The data on control experiments without inhibitors presented in this study are a subset of data previously reported in another study [12]. In brief, muscles were stabilized at L_{90} or L_{opt} for 10–15 min without any inhibitors. The k_{tr} was measured at sub-maximal and maximal tension levels of the K^+ contracture as previously described [12]. After these control measurements, muscles were re-stabilized at L_{opt}, 1 Hz, 37 °C without any inhibitors for 15 min. Muscles were then stabilized in the presence of 20 μM H-89 and 7.5 nM PKCβII peptide inhibitor I for an additional15 min. These inhibitors were also added to the high K^+/high Ca^{2+} solution (i.e. the K^+ contracture solution). The K^+ contracture was induced and k_{tr} maneuvers at sub-maximal and maximal tension levels were performed. Muscles were next stabilized at L_{90}, 1 Hz, and 37 °C with the inhibitors for 15 min. Afterwards, K^+ contracture and k_{tr} experiments were conducted during sub-maximal and maximal tension levels.

2.4. k_{tr} measurements after rapid muscle length changes

The control data at L_{opt} and L_{90} reported are from a subset of experiments previously reported in our recent study [12]. Muscles were stabilized at L_{90} and 1 Hz for ~10–15 min. K^+ contracture was induced and k_{tr} was initially measured during maximal K^+ contracture tension as described above. Immediately afterwards, while the muscles were still under contracture, they were quickly stretched to L_{opt} and k_{tr} was measured at this length quickly (within several seconds). Similarly, muscles were allowed to stabilize at L_{opt} and 1 Hz for ~10–15 min. After the k_{tr} at L_{opt} was measured during the maximal K^+ contracture tension, the muscle was quickly slacked to L_{90} while under contracture and a k_{tr} experiment was performed without stabilization (i.e. within seconds after slacking to L_{90}).

2.5. Protein analysis

For a subset of single trabeculae, total protein phosphorylation