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Titin-mediated control of cardiac myofibrillar function

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ABSTRACT

According to the Frank–Starling relationship, ventricular pressure or stroke volume increases with end-diastolic volume. This is regulated, in large part, by the sarcomere length (SL) dependent changes in cardiac myofibrillar force, loaded shortening, and power. Consistent with this, both cardiac myofibrillar force and absolute power fall at shorter SL. However, when Ca^{2+} activated force levels are matched between short and long SL (by increasing the activator [Ca^{2+}]), short SL actually yields faster loaded shortening and greater peak normalized power output (PNPO). A potential mechanism for faster loaded shortening at short SL is that, at short SL, titin becomes less taut, which increases the flexibility of the cross-bridges, a process that may be mediated by titin's interactions with thick filament proteins. We propose a more slackened titin yields greater myosin head radial and azimuthal mobility and these flexible cross-bridges are more likely to maintain thin filament activation, which would allow more force-generating cross-bridges to work against a fixed load resulting in faster loaded shortening. We tested this idea by measuring SL-dependence of power at matched forces in rat skinned cardiac myocytes containing either N2B titin or a longer, more compliant N2BA titin. We predicted that, in N2BA titin containing cardiac myocytes, power-load curves would not be shifted upward at short SL compared to long SL (when force is matched). Consistent with this, peak normalized power was actually less at short SL versus long SL (at matched force) in N2BA-containing myocytes (N2BA titin: ΔPNPO (Short SL peak power minus long SL peak power) = −0.057 ± 0.049 (n = 5) versus N2B titin: ΔPNPO = +0.012 ± 0.012 (n = 5). These findings support a model whereby SL *per se* controls mechanical properties of cross-bridges and this process is mediated by titin. This myofibrillar mechanism may help sustain ventricular power during periods of low preloads, and perhaps a breakdown of this mechanism is involved in impaired function of failing hearts.

Introduction

Muscles *in vivo* shorten against a load and, thus, generate power (which is work capacity per unit time). The velocity that muscle shortens is inversely related to the load on (or force produced by) the muscle with the relationship between force and shortening velocity generally expressed by a rectangular hyperbola [1]. Each point on the force-velocity relationship can be used to estimate power output (by simply multiplying force x velocity), where power is zero at the two extremes of the force-velocity relationship and reaches a maximum at intermediate loads. During oscillatory activity, *in vivo* skeletal muscles [2] and presumably cardiac muscle operate at intermediate forces and velocities where power is close to maximum. In the heart, ventricular stroke volume is determined by myocardial power output, which dictates the amount that the myocardium shortens against external loads arising from arterial impedance and ventricular wall stress. Since the ventricles act as a functional syncytium and presumably all myocytes are electrically activated during each heartbeat, stroke volume is determined by the power generated by individual cardiac myocytes. However, the exact mechanisms that determine power output of individual cardiac myocytes remain unanswered and have been the focus of several studies. One important determinant of myocyte power is myofibrillar sarcomere length, which is thought to underlie the Frank–Starling relationship, whereby greater end-diastolic ventricular volume increases stroke volume. We previously investigated sarcomere length dependence of loaded shortening and power output and found slower loaded shortening and less power at short sarcomere length over all absolute loads and this sarcomere length dependence persisted even when force-velocity curves were normalized for differences in isometric force, i.e., loaded shortening velocity was slower at short sarcomere length at loads less than ~40% isometric force [3]. A plausible mechanism to explain slower loaded shortening and decreased power output at short sarcomere length is the coincident decrease

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in thin filament activation levels at short sarcomere length given that force was lower due to the well described sarcomere length dependence of Ca^{2+} sensitivity of force [4]. However, when Ca^{2+} activated force was matched at short sarcomere length to those at long SL[1] (by increasing the activator [Ca^{2+}]) short sarcomere length actually yielded slightly faster loaded shortening velocities and greater peak normalized power output [3]. This suggests a myofibrillar mechanism that tends to speed loaded cross-bridge cycling to minimize the fall of power at short sarcomere length. Interestingly, treatment of myocytes with 2% dextran to compress the myofilament lattice at short sarcomere length also caused faster loaded shortening compared to long sarcomere lengths, again implicating a myofibrillar mechanism that leads to faster loaded cross-bridge cycling at short sarcomere length [3]. This finding of faster loaded shortening at *short* sarcomere length challenges classic models of cross-bridge and muscle mechanics [5] and suggests that the mechanical state of one population of cross-bridges affects the activity of other cross-bridge populations by, for example recruitment and subsequent retention of non-cycling cross-bridges into the cycling force-generating population. Physiologically, these results implicate a myofibrillar mechanism that may help sustain ventricular power during periods of lower preloads, and perhaps a breakdown of this mechanism may contribute to impaired function of failing hearts.

Our working hypothesis for faster loaded shortening at short sarcomere length (when force is matched) is that as sarcomere length is shortened, titin becomes less taut which reduces the constraint of cross-bridges, a process that may be mediated in part by titin's interaction with myosin binding protein-C (MyBP-C) on the thick filament [6] (see Fig. 1). According to this model, a slackened titin yields greater myosin head radial and azimuthal mobility [3]. This leads to faster cross-bridge cycling by creating more flexible cross-bridges that are more likely to maintain thin filament activation, which allows more force-generating cross-bridges to work against a fixed load. Importantly, though, this mechanism alone cannot overcome the decrease in the number of cross-bridges induced, in part, by increased lattice spacing that normally occurs with short sarcomere length, which is why loaded shortening is slower at short sarcomere length when activator [Ca^{2+}] is the same between long and short SL. These mechanistic ideas are consistent with findings that (i) titin binds C-terminal domains of MyBP-C [7], (ii) gene targeted ablation of MyBP-C caused radial displacement of cross-bridges away from the thick filaments [8], and (iii) ablation of MyBP-C yielded faster loaded shortening in cardiac myocyte preparations [9].

We tested titin's role in modulating loaded shortening and power output by measuring SL dependence of power at matched forces in rat skinned cardiac myocytes containing either N2B titin or the longer, more compliant N2BA titin. We predicted that a more compliant titin would augment loaded shortening at long sarcomere length leading to less overshoot in loaded shortening and power at short sarcomere length (and matched force) in N2BA titin myocytes.

We also tested the hypothesis that faster rates of force development observed at short sarcomere lengths [10–12] is mediated by titin compliance, which may affect the extent of cooperative thin filament activation. We predicted faster rates of force development in myocyte preparations containing N2BA titin due to less spread of cooperative activation along thin filament and recruitment of force-generating cross-bridges (which takes time and slows force development) following a mechanical slack-re-stretch maneuver.

[1] *Abbreviations used:* SL, sarcomere length; PNPO, peak normalized power output; MyBP-C, myosin binding protein-C.

Methods

Experimental animals

Adult mutant N2BA titin rats ($n = 9$) (which are 50% Brown–Norway, 25% Fischer, 25% Sprague–Dawley hybrid rats) [13] and control littermates ($n = 7$) were housed in groups of 2 or 3 and provided access to food and water *ad libitum*. All procedures involving animal use were performed according to the Animal Care and Use Committee of the University of Missouri.

Solutions

The compositions of relaxing and activating solutions were as follows (in mmol/L, chemicals obtained from Sigma at highest possible purity): free Mg^{2+} 1, EGTA 7, MgATP 4, imidazole 20, and creatine phosphate 14.5 (pH 7.0); specific [Ca^{2+}] between $10^{-4.5}$ (maximal Ca^{2+} activating solution) and 10^{-9} (relaxing solution); and sufficient KCl to adjust ionic strength to 180 mmol/L. Preceding each Ca^{2+}-activation, myocyte preparations were immersed for 30 sec in a solution of reduced Ca^{2+}-EGTA buffering capacity, which was identical to normal relaxing solution except that EGTA was reduced to 0.5 mM. This protocol resulted in more rapid development of steady state force during subsequent Ca^{2+} activation. Relaxing solution, in which the ventricles were mechanically disrupted and myocytes were resuspended, contained 2 mM EGTA, 5 mM $MgCl_2$, 4 mM ATP, 10 mM imidazole, and 100 mM KCl at pH 7.0 with the addition of a protease inhibitor cocktail (Set I Calbiochem, San Diego, CA).

Myocardial preparations

Skinned cardiac myocytes were obtained by mechanical disruption of hearts from rats as described previously [14]. Briefly, rats were anaesthetized by inhalation of isoflurane (20% v/v in olive oil), and their hearts were excised and rapidly placed in ice cold relaxing solution. The left ventricle was separated from the right ventricle and dissected from the atria, cut into 2–3 mm pieces and further disrupted for 5 sec in a Waring blender. The resulting suspension of cells was centrifuged for 105 sec at 165×*g*, after which the supernatant fluid was discarded. The myocytes were skinned by suspending the cell pellet for 5 min in 0.3% ultrapure Triton X-100 (Pierce Chemical Co.) in cold relaxing solution. The skinned cells were washed twice with cold relaxing solution, suspended in 10 ml of relaxing solution and kept on ice.

Experimental apparatus

The experimental apparatus for mechanical measurements of myocyte preparations was similar to one previously described [14]. Briefly, a myocyte was attached between a force transducer and high speed motor by gently placing the ends of the myocyte into stainless steel troughs (25 gauge). The ends of the myocyte were secured by overlaying a 0.5 mm long piece of 3-0 monofilament nylon suture (Ethicon, Inc.) onto each end of the myocyte, and then tying the suture into the troughs with two loops of 10-0 monofilament suture (Ethicon, Inc.). The attachment procedure was performed under a stereomicroscope (~100× magnification) using fine tipped forceps.

Prior to mechanical measurements the experimental apparatus was mounted on the stage of an inverted microscope (model IX-70, Olympus Instrument Co., Japan), which rested on a pneumatic anti-vibration table with a cut-off frequency of ~1 Hz. Force measurements were made using a capacitance-gauge transducer (Model 403-sensitivity of 20 mV/mg plus a ×10 amplifier and resonant fre-