Phosphorylation of Serine 282 in Cardiac Myosin Binding Protein-C Increases Myosin MgADP Release and MgATP Binding Rates in Mouse Myocardium

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Shortening of Titin's Elastic Tandem Ig Segment Leads to Cardiac Hypertrophy and Diastolic Dysfunction
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Diastolic dysfunction is a poorly understood but clinically pervasive syndrome. Titin is the main determinant of cellular passive stiffness. However, the physiological role that titin's tandem Ig segment plays in stiffness generation and whether shortening this segment is sufficient to cause diastolic dysfunction needs to be established by performing studies at a wide range of organizational levels (skinned cells to the in vivo LV chamber). We produced a novel KO model by deleting nine immunoglobulin(Ig)-like domains from the proximal tandem Ig segment (Ig 3-11) of titin's spring region (IG KO). Exon microarray analysis revealed no adaptations in titin splicing, while novel phospho-specific antibodies did not detect changes in titin phosphorylation. Passive myocyte stiffness was increased in the KO and immunoelectron microscopy revealed increased extension of the remaining titin spring segments as the likely underlying mechanism. Diastolic stiffness was increased at the tissue and organ levels; myocardial stiffness studies did not detect changes in extracellular matrix based passive stiffness, supporting a titin-based mechanism. IG KO mice have a reduced exercise tolerance and develop LV hypertrophy that is associated with a marked increase in expression of hypertrophy-associated four and a half LIM proteins (FHL). These findings suggest that titin-based stiffness plays a role in diastolic dysfunction and hypertrophic signaling and that increased titin-based passive stiffness is sufficient to cause diastolic dysfunction with exercise intolerance and LV hypertrophy.

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Titin Mediates Sarcomere Length Dependence of Myocyte Power
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According to the Frank-Starling relationship, ventricular pressure increases with end-diastolic volume. This is controlled, in large part, by sarcomere length (SL) dependence of cardiac myofibrillar force, loaded shortening, and power. Consistent with this, both myofibrillar force and power fall at shorter SL, however, when Ca^{2+} activated force and presumably thin filament activation 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 as SL decreases titin becomes less taut which reduces the impedance of cross-bridges, a process that may be mediated by titin's interactions with the thick filament. 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 less overshoot in power by short SL in N2BA titin myocytes. Consistent with this, peak power did not overshoot but was actually less at short versus long SL at matched thin filament activation levels in N2BA-containing myocytes (Wt: ΔPNPO = $+0.0121 \pm 0.0115$ (n=5), N2BA titin: ΔPNPO = -0.0566 ± 0.0493 (n=5)). These findings are consistent with SL *per se* modulating the mechanical properties of cross-bridges with this modulation being 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.

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Cardiac Myosin Binding-Protein C (cMyBP-C) Phosphorylation affect Cross-Bridge Function
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To understand the functional significance of phosphorylation that takes place in the M domain of cMyBP-C, chemically skinned papillary muscle fibers of transgenic mice were studied by sinusoidal length alterations and concomitant tension transients. Muscle fibers were maximally activated at pCa 4.55 in the solution that mimic physiological conditions (5 mM MgATP, 8 mM Pi, 200 mM total ionic strength with K-acetate) in myocytes. WT mice possess phosphorylation sites S273, S282, and S302. SAS is a single mutant S282A, and ADA and DAD are triple mutants S273A/S282D/S302A and S273D/S282A/S302D, respectively. D models for phosphorylation (phosphomimetic), and A models for non-phosphorylation (phosphor-ablation). Isometric tension and stiffness of DAD were respectively ~0.5x of those of WT, but tension and stiffness of t/t (cMyBP-C null), ADA, and SAS were respectively similar to WT. The fast rate constant $2\pi c$ of DAD and t/t was ~0.6x of WT, but that of ADA and SAS was similar to WT. The intermediate rate constant $2\pi b$ of DAD and SAS was ~1.3x of WT, but that of ADA and t/t was similar to WT. These results demonstrate that cMyBP-C M domain phosphorylation affects the cross-bridge kinetics at ATP binding and phosphate release steps, indicating that phosphorylation affects myosin structure and its interaction with actin. However, pCa-tension and pCa-stiffness studies demonstrated that pCa_{50} (Ca^{2+} sensitivity) and n_H (cooperativity) were respectively not different among mutants and WT groups, indicating that phosphorylation of cMyBP-C has a minimal effect on the regulatory system. The decreased amount of isometric tension only in DAD indicates that phosphorylation of S273 and S302 are most significant and they diminish the force generation capability, presumably owing to the extra electrostatic interaction of the M domain of cMyBP-C with actin thin filament, which may serve as a break.

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Phosphorylation of Serine 282 in Cardiac Myosin Binding Protein-C Increases Myosin MgADP Release and MgATP Binding Rates in Mouse Myocardium
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Cardiac myosin binding protein-C (cMyBP-C) modulates contractility in part through phosphorylation of serines (S) found in the cardiac-specific motif between domains C1 and C2. S282 phosphorylation appears to allow the hierarchical phosphorylation of S302 and S307 by protein kinase-A (PKA). We investigated whether S282 phosphorylation status influenced the effects of PKA on acto-myosin cross-bridge MgADP release rate (k_{-ADP}) and MgATP binding rate (k_{+ATP}) in myocardial strips. Transgenic mice expressing cMyBP-C with a single amino acid switch at S282 to glutamic acid (D) or alanine (A) were generated to mimic phosphorylated or unphosphorylated states, respectively. Mice were fed L-thyroxin for 10 days to ensure similar (>90%) α-myosin heavy chain (α-MyHC) background. Skinned papillary muscles were pretreated with alkaline phosphatase to reduce any differential *in vivo* phosphorylation, and examined by length perturbation analysis at maximum calcium activation (pCa 4.8) as MgATP concentration varied (0 to 5 mM). α-MyHC k_{-ADP} and k_{+ADP} increased 90% (212 ± 22 *vs.* 111 ± 9 s^{-1}) and 45% (867 ± 93 *vs.* 595 ± 50 mM^{-1} s^{-1}) for S282D *vs.* S282A, demonstrating that cMyBP-C S282 phosphorylation status significantly affects α-MyHC kinetics. Following PKA treatment, k_{-ADP} and k_{+ADP} values did not differ between S282D and S282A, due to PKA reducing k_{-ADP} by 23% (163 ± 19 s^{-1}) and k_{+ATP} by 45% (475 ± 35 mM^{-1} s^{-1}) for S282D, but increasing k_{-ADP} by 23% (137 ± 9 s^{-1}) and decreasing k_{+ATP} by 23% (457 ± 52 mM^{-1} s^{-1}) for S282A. These results suggest that S282 phosphorylation most significantly affects the initial functional status of the myocardium, prior to PKA-mediated effects from cMyBP-C or other phosphorylatable myofilament proteins.

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Activation and Inhibition of F-Actin and Cardiac Thin Filaments by the N-Terminal Domains of Cardiac Myosin Binding Protein C
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Myosin binding protein-C (MyBPC) has been known for over thirty years to inhibit actomyosin ATP hydrolysis and more recently has been shown to affect the calcium sensitivity of force in muscle fibers. The various domains of MyBPC have previously been shown to have complex interactions with other myofibrillar proteins. The C-terminal domains have been shown to bind to the thick filament and the N-terminal domains interact with the S2 region of myosin and with f-actin. The number of mutations in cardiac MyBPC (cMyBPC) are the second most prevalent of sarcomeric proteins in producing cardiomyopathies. We have used steady state kinetics to study the molecular mechanism by which the soluble N-terminal C0C1, C1C2 and C0C2 domains of mouse and human cMyBPC affect the activation of myosin ATP hydrolysis by f-actin and native porcine thin filaments.