The mechanisms of the Frank-Starling law and familial cardiomyopathy are different. The function of myosin binding protein-C is retained on myocyte length increase and force generated is kinase controlled.

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Summary.

I have recently reiterated that the cross-bridge is a calcium ATPase that is inhibited by magnesium and this arises because in normal hearts Myosin binding Protein-C prevents the use of MgATP as rate limiting substrate ensuring that Ca^{2+} replaces Mg^{2+} in the excitation pathway. Here I revisit the studies on $[Ca^{2+}]$ dependency of ATPase and tension under diastolic stretch with a different conclusion on Hill coefficients. This reveals the underlying mechanisms of the Frank-Starling Law and Hypertrophic myopathy are not the same, the former being kinase controlled.

The function of myosin binding protein-C.

As demonstrated by Solaro[1,2] Mg^{2+} is a competitive inhibitor of the Ca^{2+} stimulation of cardiac muscle ATPase and contraction. On the basis of the measured binding affinities[1,2] I have argued that the inhibition is not via the troponin-C binding of Ca^{2+} but via a second transient effector binding site[3,4] dependant on ATP and hence not measurable by $^{45}Ca^{2+}$ binding and proposed that this is the myosin-bound ATP or the myosin bound products MgADP and Pi or transient phosphoprotein. The Myosin binding Protein-C (MyBP-C) makes Ca^{2+}-Mg^{2+} exchange or a consequent step rate-limiting in cross-bridge cycling and prevents MgATP appearing as the apparent substrate[3]. This is consistent with both the contraction effective Ca^{2+} affinity being lower than that of troponin-C and the elevated Ca^{2+} cooperativity observed for both myofibril ATPase and tension development (Hill coefficient 2 in normal heart tissue).

On dissociation or removal of MyBP-C and in hypertrophy the Hill coefficient is greatly reduced and the activating $[Ca^{2+}]$ approaches the affinity of Troponin C[5,6], i.e. MgATP becomes the rate-limiting substrate of the cross-bridge ATPase.

On diastolic stretch the function of MyBP-C is retained.

Data taken from various sources indicates that under the effect of length increase during diastole, the effective Ca^{2+} affinity for tension development rises a little towards that of troponin-C[7,8,9,10,11], much less than when MyBP-C is

removed[5,6]. This is without a significant decrease in Hill coefficient, my earlier treatment was wrong[12,13], I admit quoting an incorrect review article and apologize. Here reports with a highly elevated Hill coefficient in the unstretched state were rejected as mechanical cooperativity would swamp any chemical cooperativity. There is one case where the ATPase activity associated with stretch has been investigated[14] and, although the resting Ca^{2+} Hill coefficient is a bit less than 2, it shows a small shift to higher Ca^{2+} affinity with no significant reduction in the Hill coefficient. Whether measured as tension or ATPase activity under diastolic stretch, the MyBP-C retains its biochemical function, i.e. inhibiting MgATP as rate-limiting substrate[3].

The current understanding of length induced change.

After Moss[15] there has been little advance since the review of myofilament length dependent activation by de Tombe et al[16]. The above is consistent with there being a critical dimension in the sarcomere of cardiac muscle that is altered with length increase applied in diastole. It is possible but unlikely that this dimension is the separation of the thick and thin fillaments[10,11]. As there is no change in the Hill coefficient on shift to lower $[Ca^{2+}]$ it is the result of simply raising the effective Ca^{2+} affinity of either the troponin-C[1,2,8,17.18,19] or the myosin bound ATP/ADP[3,4] , i.e. increased exchange rate of Ca^{2+} for Mg^{2+}.

Relationship to cardio myopathy.

It has been noted by us[12] and others cited in the same reference that there are correlations between studies on the Frank-Starling law and those on familial hypertrophic cardiomyopathy in that they both show a shift to activation at lower $[Ca^{2+}]$ although as reported here only the hypertrophy shows a large shift and definite lowering of the Ca^{2+} cooperativity[1] as is also shown on removal of MyBP-C[5,6].

In many instances of the reverse of hypertrophy, i.e. familial dilated myopathy, there is also the noted absence of the effect of diastolic stretch on the strength of contraction. In dilated myopathy there is always complete relaxation in diastole and much reduced tension in systole resulting in a deficiency in muscle growth, vide infra. These myopathies are mostly arising from mutations in the Troponins[18] suggesting that the effect of normal tension maybe in part mediated via troponin and the thin filament.

The above correlation led us to believe that in familial hypertrophic cardiomyopathy the effect of any one of the associated mutations (in genes encoding for sarcomeric proteins[20] mostly MyBP-C or myosin) was to render the normally inhibitory Mg^{2+} to be stimulatory[3]. The most significant result of these mutations is loss of Ca^{2+} cooperativity rendering the relaxation in diastole incomplete resulting in a state of chronic tension. It is proposed that chronic

tension allows time for a degree of unfolding of the elastic portion of titin[21,22] and the release of nuclear activating factors, e.g. muscle LIM protein, that are bound to the same elastic region. The release of the activating factors promotes inappropriate growth of the sarcomere and the resulting disarray of muscle fibres observed in most cases of familial hypertrophic myopathy.

This is not the mechanism of Frank-Starling activation as full relaxation occurs with diastolic length increase, i.e. the Ca^{2+} Hill coefficient of activation is not reduced, the MyBP-C function is retained.

The involvement of kinases in the Frank-Starling Law.

The elastic giant protein titin binds myosin and MyBP-C and their involvement via altered myosin heads interaction with the thin filament, is still a contender for length induced changes.

However a recent study with structural sensitive probes indicates that changes in the myosin head region[23] are responsible for the stretch induced amplification of maximal tension with minimal changes in the thin filament.

By the use of knock-out mice the misnamed "multifunctional" transducer protein β-Arrestin has also been shown to be essential to produce the length induced tension increase and shift towards the troponin Ca^{2+} sensitivity through the angiotensin type 1 receptor[24], but not via the phosphoinositide-Ca^{2+}second messenger system, i.e. no change in resting or stimulated $[Ca^{2+}]$ is involved.

The presence of cardiac troponin-I (cTn-I) is also a requisite for length induced activation[16] and is possibly a substrate for kinase-C or MLCK action as only the threonine at position 144 in cardiac TnI is essential, the serines are not. This is shown by substituting a hybrid skeletal Tn-I, missing serines, with added threonine at residue 144.

Protein phosphorylation[25] does though appear to be involved, in the case of MyBP-C and its insistence on $Mg^{2+} - Ca^{2+}$ exchange one would expect acceleration of the rate determining step as is found on posphorylation. Although kinase action on the troponins does modulate contraction strength coupling with length induced changes has not been directly demonstrated. β-Arrestin stimulated Ca^{2+}-independent phosphorylation has also been muted but again not shown (does not exist?).

Losartan[24] a drug that blocks angiotensin II type 1 receptor (AT1R) activation is found to block the length induced enhancement of cardiac sensitivity. So on length increase of the plasma membrane (PM) it would seem the AT1R appears activated.

The next steps in the sequence are phosphorylation of the activated AT1R tail by adrenergic receptor kinase (GRK) followed by translocation of β-Arrestin from the cytoplasm to bind strongly to the phosphorylated AT1R[26,27] at the plasma membrane. This binding inhibits further phosphorylation of the AT1R, β-Arrestin is a kinase inhibitor!! From where its name originates. No way does it change its spots with cell location.

Myosin light chain kinase (MLCK) activity[28,29,30], would fit very well with the structural changes reported in the myosin heads, see above[23]. It is constitutively active, is Ca^{2+} and calmodulin dependent but has 38% basal activity in absence of calmodulin[30] and in vivo is associated with a calmodulin inhibitor. It is almost certainly inhibited by the β-Arrestin normally in the sarcoplasm, removal of the inhibition occurs on angiotensin activation or as we see here by stretch. This does not preclude similar Kinase A or C (PKA or PKC) activation.

Myosin light chain phosphorylation by PKA, PKC or MLCK will increase the affinity of the myosin bound ATP/ADP for Ca^{2+} over that for Mg^{2+}, more negative charge and chelating phosphate groups, shifting the sensitivity to lower $[Ca^{2+}]$ and increasing contractile strength. This is supplemented by Tn-I phosphorylation at residue 144, lowering its inhibitory role[16].

The relationship of the above to normal Cardiac function.

As with the phosphorylations above[25] the amplitude of tension has been shown by de tombe et al to be dependent on phosphorylation by PKA of both MyBP-C and Troponin-I[31]. Similar results were found with PKA phosphorylation of MyBP-C by Steltzer et al[32]. However these results could also arise through the above mechanism, distinguishing one kinase activation in the presence of all is not a trivial matter. The functions of and regulatory role of MLC is well documented[33] and control of these in general cardiac function is also documented[34,35].

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