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Calcium, actomyosin kinetics, myosin binding protein-c and hypertrophic cardiomyopathy

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Abstract

In an attempt to correct misunderstandings this article brings together the observations on Calcium, Myosin Binding Protein-C and Hypertrophic Cardiomyopathy in the basic function of cardiac muscle. A finding of many years ago is reiterated in a novel enzyme kinetic format with defined rate limiting step which makes CaATP the apparent substrate of the actomyosin cross-bridge. The relationship of these kinetics to recent observations on disruption of myosin binding protein-C is described along with how this bears on the understanding of the related cardiomyopathies.

The roles of Calcium and Adenosine triphosphate (ATP)

Based on our earlier publication [1], I have constructed simple derivation of the 'CaATP as cross-bridge substrate proposition' in terms of enzyme kinetics and present it here, For normal cardiac muscle the well-established accepted facts are, a) The myofilament ATPase dependency on Ca^{2+} shows cooperativity, i.e. a Hill coefficient of 2 indicating probable two site binding of activating Ca^{2+} [2-5]. b) The Ca^{2+} stimulated ATPase shows competitive inhibition by Mg^{2+} [3,4]. c) The primary binding site for Ca^{2+} activation is troponin which is measurable by $^{45}Ca^{2+}$ binding. This binding to troponin removes its blocking of the thin filament actin allowing the thick-filament myosin to form a cross-bridge to the actin [3-5]. d) The cross-bridge consumes ATP to generate contraction and/or tension in the muscle [6]. The ATP Hill coefficient of the ATPase is 1 i.e. unimolecular. e) The troponin binding site shows no affinity for Mg^{2+} so it is not the competitive Mg^{2+} inhibition site, this confirms the existence of a transient second Ca^{2+} binding site that is competitively inhibited by Mg^{2+}, but not measurable by $^{45}Ca^{2+}$ binding, i.e. requires ATP.

f) The very simple conclusion is that in the unperturbed heart the cross-bridge is a Ca^{2+} ATPase and inhibited by Mg^{2+}, i.e. MgATP binding to the myosin gives the relaxed state ready for Mg^{2+}-Ca^{2+} exchange and cross-bridge cycling if the troponin is still Ca^{2+} bound. This is consistent with the Ca^{2+} cooperativity, i.e. Hill coefficient 2. It is clear from two studies [7,8], one very recent, that replacement of the product CaADP from the cross-bridge with MgATP is essential to complete the lever arm cycle.

There is one caveat to add to this scheme. For this kinetics to be true the replacement of Mg^{2+} with Ca^{2+} or a subsequent step dependent on this has to be the rate determining step. It is possible that the pyrophosphate bond is not hydrolyzed but a phosphate group is transferred to a protein (myosin light chain?), increasing the charge carried and thus raising the Ca^{2+} affinity of the ADP bound protein above that of Mg^{2+}. This clearly happens with phosphorylation by myosin light chain kinase [9].

Myosin binding protein-C (MyBP-C)

Irving et al. [10] have shown that dissociation of MyBP-C from myosin by addition of its binding fragment C1mC2 reduces the Hill coefficient for Ca^{2+} activation to unity and shifts the Ca^{2+} sensitivity to the affinity of troponin-C. This is also found by Hofmann et al. [11] on the reversible physical removal of the MyBP-C. The sole disruption of C1mC2 is to the myosin binding of MyBP-C as is the reversible physical removal of it, leaving the Ca^{2+} troponin binding being the sole activator, i.e. Ca^{2+} Hill Coefficient 1. These procedures both remove the necessity of Ca^{2+} replacement of the Mg^{2+} that is bound with ATP to the myosin for cross-bridge cycling to occur, i.e. MgATP use becomes the rate limiting step. It is not known if the Ca^{2+}-Mg^{2+} still occurs but probably not. The conclusion from this is that the function of myosin binding protein-C is to ensure that MgATP is not the apparent substrate for cross-bridge cycling in the unperturbed normal heart and the Ca^{2+}-Mg^{2+} exchange defines the rate limiting step of the actomyosin ATPase. Thus, maintaining the full cooperativity of Ca^{2+} activation and normal Ca^{2+} sensitivity well below the Troponin affinity and hence full diastolic relaxation, see later. This is the first recorded biochemical function of MyBP-C although its phosphorylation is recognized as an important regulator in muscle function.

It has previously been accepted that MyBP-C is a key structural protein of the thick filament but being so easily reversibly removed [11] this is not so. It has an affinity for the thin filament, but this is displaced by the myosin.

Hypertrophic cardiomyopathy

Cardiomyopathy are diseases that to variable debilitating extent affects some 1 in 200 of the world's population. Hypertrophic cardiomyopathy, often undiagnosed as it appears as a healthy heartbeat in earlier stages, does eventually give problems with high diastolic and

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systolic pressure. What is more is one often hears of young people especially athletes and sportsmen experiencing sudden cardiac death as a result of the condition, when the conductance of their heart is extremely faulty resulting from the myofilament disarray of severe hypertrophy.

Hugh Watkins [12] and others [13,14] cite various mutations in genes encoding for sarcomeric proteins, mostly MyBP-C and myosin, that are a common cause of hypertrophic cardiomyopathy. This is as a result of muscle growth arising from incomplete relaxation between beats, i.e. sustained tension [15,16], when the Ca^{2+} cooperativity is reduced and Ca^{2+} sensitivity greatly increased by mutated MyBP-C or myosin (MgATP reaction is then rate determining, as happens in vitro when disabling myosin MyBP-C binding to the myosin) [10,11]. The elastic giant protein titin binds myosin and MyBP-C. It has been proposed that sustained tension allows time for a degree of unfolding of the elastic portion of titin [15,16] and the release of nuclear activating factors, e.g. muscle LIM protein, that are bound to the 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.

Non-medication of hypertrophic cardiomyopathy

For those that have hypertrophic myopathy diagnosed as a result of heart problems there is only lifestyle change recommended. A drug to increase relaxation rate is required to prevent further sustained tension mediated growth, levosimendan seems to do this but also increases the contraction rate and so would need to be used in conjunction with a Ca^{2+} uptake inhibitor to reduce the latter. It is interesting that this drug combination has been administered successfully when levosimendan has been used during rescue of an attempted suicide patient [17].

Footnote

Most of the above save the cardiac diseases, applies equally to mammalian skeletal muscle, with differences in isoforms of the constituent proteins.

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