New Concepts in the Control of Muscle Contraction

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Gerry is a synthetic organic chemist who has collaborated in many biological fields ranging from pharmaceutical synthesis at Glaxo Research through synthesis of bioactive molecules, structural studies, e.g. Vancomycin, lipid-protein interactions and more recently antibiotic and anti-cancer drugs.

This life of chemical service to biology is not as mundane as first appearances would indicate. In just one field alone, the synthesis of cryptand based [Na^+] indicators, the design of the most selective binding site for Na^+ over K^+ was required. During this work a completely novel synthesis of one heterocycle was discovered along with the need to add sodium ions to a $ZnCl_2$ (Lewis acid) catalysed condensation and most surprising of all the addition of water was found essential for a rapid and clean borane reduction of cyclic amides to amines.

The main theme of research over the last few years has been the spectroscopic measurement of cation concentrations, [H^+], [Na^+], [Mg^{2+}], [Ca^{2+}]. From the chemist's viewpoint it is essential to follow through from the synthesis of the tools into their use in the biological sphere. This is to ensure that rigorous chemical and physical principles are applied during the collection, and analysis, of the resulting data. This is exemplified by the series of papers initiated by the synthesis of ^{19}F-NMR indicators and their use in the Langendorff-perfused heart. This series of eight papers is contained within this book, wherein a logical physical-chemical approach leads to an understanding of a considerable amount of unexplained, and thus often ignored, observations on cardiac contractility and drug action.

Preface

This is a collection of papers that presents a novel interpretation of data from the literature to reason logically for an overlooked mechanism of stimulus-contraction coupling in muscle. This mechanism is then used to explain aspects of the puzzles relating to both an important physiological function of the heart, The Frank–Starling Law, and the basis of a common inherited disease state, familial hypertrophic cardiomyopathy (FHCM).

The first three papers were published in the Biochemical Journal and are reproduced here by permission of the Biochemical Society Editors (Portland Press). The remaining five papers are an examination and reinterpretation of data in papers that are already published. Great care has been taken to attribute correctly these data to the publications and their authors. These latter papers have failed to get past the submission offices of a number of serious journals and thus will now have peer review in the public domain.

Many published studies in this field have been excluded from subsequent consideration because they do not fit within the established "solely troponin-Ca^{2+}-binding activated, single-headed myosin, multiple cross-bridge highly cooperative model of muscle contraction". The purpose of the current study is to remove the words "solely" "single-headed" "multiple … highly cooperative" from this sentence to reveal the more simple;

"a cross-bridge model of muscle contraction activated both
by calcium binding to troponin and by calcium binding to myosin".

This encompasses the established principles of muscle stimulus-contraction coupling but adds the thick-filament activation that has been mooted often in the literature but never attributed a mechanistic basis.

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Introduction

This series of papers was initiated by the availability of a range of Ca^{2+} indicators whose degree of saturation changes the nuclear magnetic resonance of the ^{19}F they contain. Having differing dissociation constants and resultant Ca^{2+} buffering ranges, they have allowed us to extend the measurement of cytosolic [Ca^{2+}] in the intact beating heart. This methodology has overcome the age-old bugbear of science where the method used perturbs the target to be measured.

The first Paper **(I)** in this series [previously reported in the Biochemical Journal (2000)] entitled: **Estimation of systolic and diastolic free intracellular Ca^{2+} by titration of Ca^{2+} buffering in the ferret heart.** Loading hearts with indicators resulted in the expected variation of buffering of the Ca^{2+} transient. The measured end-diastolic- and peak-systolic-$[Ca^{2+}]_i$ were both positively correlated with indicator dissociation constant (K_d). These correlations were used to estimate the unperturbed values by extrapolation to $K_d = 0$ (diastolic-$[Ca^{2+}]_i$) and to $K_d = \infty$(systolic- $[Ca^{2+}]_i$) because indicators with these K_d values will be fully bound and fully free under all conditions and hence not act as buffers.

The extrapolated values in the intact beating heart are the first reported reliable end-diastolic- and peak-systolic-$[Ca^{2+}]_i$ measurements. With some minor assumptions, knowing these values and those actually observed in the presence of known concentrations of the various indicator-buffers has allowed access to calculation of the relative buffering capacity of the myofibrils in the intact heart. This second Paper **(II)**, **Ca^{2+} buffering in the heart: Ca^{2+} binding to and activation of cardiac myofibrils**, accompanied the previous Paper (I) in the Biochemical Journal (2000). The generally used concentrations of other spectroscopic indicators, e.g. Fura-2, also have a massive effect on the Ca^{2+} transient. During preparation of this paper it became obvious that the simple model of activation by binding of Ca^{2+} to troponin-C was not quite sufficient to explain the data. Incorporation of an ATP-dependent additional transient Ca^{2+}-binding, as indicated by the work of Morimoto and Ohtsuki (1994, see Paper (II) reference section) not only improved the modelling fit but also produced Ca^{2+} and ATP affinities that closely predicted the Ca^{2+}-ATPase activity of myofibrils measured *in vitro*.

At this stage of the studies the large amount of data (e.g. those from Solaro 1976, see Paper (III) reference section) relating to the inhibitory effects of Mg^{2+} on the myofibrillar ATPase and related came to the authors' attention. These data on the effects of the concentration of [Mg^{2+}], along with related effects found in studies of the poorly explained mechanisms of both the Frank–Starling Law and familial cardiac myopathies, have not yet been absorbed into mainstream muscle research. The lack of a known Mg^{2+} binding of relevant strengths, that is measurable by static methods, most probably underlies the current position.

The third of the published papers in this series **(III)** is to date the only serious attempt to marry the Mg^{2+} inhibition with the accepted troponin-C based mechanism of myofibril activation. This work is entitled, **The effect of Mg^{2+} on cardiac muscle function; is CaATP the substrate for priming myofibril cross-bridge formation and Ca^{2+} re-uptake by the sarcoplasmic reticulum?** The earlier work above (II), indicated two Ca^{2+}-binding sites, possibly one for each paired myosin head, to act as a single unit in initial cross-bridge formation. A large positive inotropic (contraction-intensifying) effect of reducing the cytosolic [Mg^{2+}] by introduction of a suitable chelator, citrate, confirmed the competitive inhibition of the Ca^{2+} activation by Mg^{2+}, previously seen *in vitro*. In the absence of a recognized second Ca^{2+}-binding site on the myofibril, with appropriate binding properties, the myosin-bound ATP is proposed as the second activating (cross-bridge recruiting) Ca^{2+}-binding site. Published physico-chemical studies on skeletal muscle have shown that CaATP is potentially a more effective substrate than MgATP for cross-bridge formation. Calculations from this model show the Mg^{2+} effect on Ca^{2+}-dependency and its associated changes of Hill coefficient. Also explained is the burst ATPase, i.e. the slowing of phosphate release occurring in second and third cycles of ATPase.

The specific requirement for phosphocreatine (PCr) for full ATPase activity *in situ* and its effect on the apparent affinity of the myofibrils for ATP is simply a result of limited diffusion rates within the myofibrils and the relative Mg^{2+}-binding affinities of PCr, phosphate and creatine contrasted with those of ATP and phosphoenolpyruvate and their hydrolysis products.

The first previously unpublished paper in this series **(IV)** is entitled; **The Frank–Starling Law of the Heart; cross-bridge recruitment and Ca^{2+} cooperativity.** This Paper starts with the insight made by the author and his colleagues into the mechanism of the control of cross-bridge recruitment by the little understood Mg^{2+}-inhibited Ca^{2+} binding to the myofibril. This is then used to rationalize the many observations made in the literature relating the effect of changes in the concentrations of reactants and products of the actomyosin ATPase activity on the level of increased contractile strength resulting from stretch of cardiac muscle in diastole. We concluded that stretch in diastole, i.e. where there are no cross-bridges present, results in abrogation of the need for the Mg^{2+}-inhibited, non-troponin-C related, Ca^{2+} binding for activation.

Direct evidence is cited to demonstrate the reduced Ca^{2+} binding related to activation and the resultant slowing of the decay of cross-bridges during the relaxation.

The Ca^{2+} cooperativity observed in contraction is central to the logical arguments used in reaching the conclusion in Paper (IV) and the theoretical base of this is expanded in Paper **(V)**, a purely biochemical kinetics paper; **Dual Ca^{2+} Site Activation of Cardiac Muscle Contraction; Experimental isolation of the Ca^{2+} controls.** Here evidence against long-range cooperativity of both cross-bridges and multiple troponin-C Ca^{2+} binding is presented. A fundamental biochemical kinetic study is cited to show the most likely origins of the Ca^{2+} cooperativity in the actomyosin ATPase. Furthermore, studies are cited that demonstrate the independent and reversible removal in turn of both the troponin-C based Ca^{2+} control (thin-filament activation) and the Mg^{2+}-inhibited Ca^{2+} control (thick-filament activation). A conclusion is drawn that the Mg^{2+}-inhibited Ca^{2+} binding is overriding in limiting cross-bridge recruitment as a result of having a much lower affinity than that of troponin-C. The biochemical relationship of the kinetic model to the Frank–Starling Law (1915, see Paper (IV) reference section) is further strengthened, i.e. the thick-filament activation is by-passed by diastolic stretch.

The above leads directly to the question posed in the short review Paper **(VI)**; **The Frank–Starling Law of the Heart; Coupling of stretch to substrate change: A Review** This demonstrates the current state of confusion in the literature regarding the mechanism. Evidence exists both for and against a relationship between the stretch-induced change in separation of the myofilaments and the strength of contraction. However, it is clear that some change in a sarcomeric dimension has to be involved in considerations of the Frank–Starling Law. These questions are resolved in the next Paper, see below.

At this stage the question arises of whether there is a relationship between the new insight into the Ca^{2+}/Mg^{2+} control of cardiac muscle contraction and the common inherited disorder familial hypertrophic cardiomyopathy (FHCM). This question leads to Paper **(VII)** in the series; **The Frank–Starling Law and Familial Hypertrophic Cardiomyopathy; Some new striking aspects of muscle structures.** This disease state (FHCM) is odd in that it results from any one of around 200 mutations spread over a dozen or so genes that encode for the proteins of the sarcomere. The outstanding candidate as the unifying root cause of this syndrome has to be within the dimensions of the sarcomere; nothing else is likely to be commonly affected by so many different changes in so many different proteins. The relationship of both Ca^{2+} sensitivity and cooperativity changes brought about by FHCM and diastolic stretch add strength to the sarcomeric dimension ideas. Commonly cited electron micrograph data, biochemical composition data, X-ray structural studies and differences between skeletal and cardiac muscle lead to the inevitable conclusion that the previously used model of muscle structure included a major error.

It is concluded that for mammalian muscle there are two types of thin filament associated with each thick filament, one attached to the Z-band and C-region, the other being shorter and only strongly associated with the C-region. The differences between muscle types rest, along with isomeric protein forms, e.g. nebulin in skeletal and nebulette in cardiac, largely in the relative lengths of these two types of thin filament. In cardiac muscle the thin filaments that are not attached to the Z-band are, along with the nebulette, too short to span the full length of the cross bridge forming C-region through the i-region to the Z-band. Therein lies the fixed resting length peculiar to the cardiac sarcomere and hence the effect of stretch. We suggest that this critical dimension is also at the root of FHCM.

The above studies allow the construction of a biochemical kinetic model to test further possible predictions. This is presented in Paper **(VIII)**; **Biochemical Kinetic Calculations of Dual Ca^{2+} Site Activation of Cardiac Muscle Contraction; The mechanisms of effectors.** In this paper a biochemical kinetic model is constructed (refined from that previously given by the author). A new approach is used to correlate ATPase activity to rigidity and the resultant force of contraction. Generally accepted literature kinetic constants and affinities are used to establish the control situation. Application of the known $[Ca^{2+}]$ transient predicts a contractile strength that is approximately 20+% of maximum, in agreement with the increases inducible by stretch or drug treatment. Which kinetic constants are altered by the given perturbations are shown along with expected and found changes in sensitivity to Ca^{2+} and the cooperativity involved. For example, the phosphorylation of troponin-I by adrenergic stimulation has, as observed, a reverse effect to that expected, the myofibril becomes less sensitive to Ca^{2+} concentration with reduced cooperativity, but also results in the necessary faster relaxation. A major finding is that increases in contractile strength induced by Levosimendan are a result of promotion of thick filament (CaATP) activation of cross-bridge recruitment that is manifest as a small reduction in static $^{45}Ca^{2+}$ binding to the myofilaments, contrary to belief that the drug's action is a result of increased Ca^{2+}-troponin-C binding cooperativity.

The work presented promotes novel approaches to the understanding and treatment of many heart conditions.