Angiotensin II type 1 receptor and the activation of Myosin Light-Chain Kinase and Protein Kinase C-βII: Mini Review

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Summary

The involvement of the angiotensin II type 1 receptor in the Frank-Starling Law of the Heart, where the various activations are very limited, allows simple analysis of the kinase systems involved and thence extrapolation of the mechanism to that of angiotensin control of activation of cardiac and skeletal muscle contraction. The involvement of phosphorylation of the myosin light chain in the control of contraction is accepted but not fully understood. The involvement of troponin-I phosphorylation is also indicated but of unknown mechanism. There is no known signal for activation of myosin light chain kinase or Protein Kinase C-βII other than Ca²⁺/calmodulin, but the former is constitutively active and thus has to be under control of a regulated inhibitor, the latter kinase may also be the same. Ca²⁺/calmodulin is not activated in Frank-Starling, i.e. there are no diastolic or systolic [Ca²⁺] changes. I suggest here that the regulated inhibition is by myosin light chain phosphatase and/or β-arrestin. Angiotensin activation, not involving G proteins. is by translocation of the β-arrestin from the sarcoplasm to the plasma membrane thus reducing its kinase inhibition action in the sarcomplasm. This reduced inhibition has been wrongly attributed to a mythical downstream agonist property of β-arrestin.

Frank-starling law of the heart

It will seem strange to initiate this study with the following full consideration of the Frank-Starling law of the Heart (FSLH). FSLH is an increase in contraction with increased ventricular filling, i.e. increase in contraction on increase in myofibril length in diastole. The involvement of secondary factors on myocyte length increase activation is very limited allowing correct attribution of functions. For example Van der Velden, et al. [1], suggest that sarcomere length dependence of calcium sensitivity and the effects of phosphorylation by protein kinase A (PKA) of troponin-I (cTn-I) and myosin binding protein-C (MyBP-C) are independent. Steinberg [2] reports that protein kinase C (PKC) activation via Ca²⁺/calmodulin would reduce contractile strength and no changes in lipid based activators are reported on stretch. Supporting this Chandra, et al. [3] report that PKA phosphorylation has a negative effect on Ca²⁺ sensitivity but they get mixed up with the weak binding of Mg²⁺ to the troponins which has absolutely no effect on the activation of the cross-bridge. Kobayashi [4] also reports minimal effects of mimics of PKC phosphorylation of cardiac troponin-I (cTn-I). Neither of the common PKA or PKC take part in FSLH.

The chemistry of contraction

My recent study [5] of the mechanism of FSLH led me to reconsider the Ca²⁺ cooperativity of contraction of an unperturbed heart [6] and its inhibition [7] by Mg²⁺. The conclusion of this was that the cross-bridge ATPase is dependent on Ca²⁺ binding at two sites. In normal muscle the cross-bridge is restricted by myosin binding protein-C (MyBP-C) to only use CaATP as apparent substrate, i.e. the myosin-ATP bound Mg²⁺ has to be exchanged with Ca²⁺ for the cross-bridge to function. The consequential rate limiting step defined by Lionne [8] and Lynm [9] is release of phosphate which indicates phosphate is initially reversibly transferred to the myosin light chain.

\[ \text{MgATP-myosin} \rightleftharpoons \text{MgADP-phosphomyosin} \]

The exchange of Mg²⁺ for Ca²⁺ follows.

\[ \text{Ca}^{2+} + \text{MgADP-phosphomyosin} \rightleftharpoons \text{CaADP-phosphomyosin} + \text{Mg}^{2+} \]

The lever arm action follows and back to the resting state on binding MgATP.
CaADP-phosphomyosin $\rightarrow$ CaADP-myosin + Pi

MgATP + CaADP-myosin $\rightarrow$ MgATP-myosin + CaADP

The phosphate (Pi) release may be dependent on the MgATP binding.

The requirement of both the exchange of Ca$^{2+}$ for Mg$^{2+}$ and the cross-bridge initiation by Ca$^{2+}$ binding to troponin-C (Tn-C) [10,11] makes the Ca$^{2+}$ activation of the cross-bridge cooperative [5] i.e. the Hill coefficient for Ca$^{2+}$ activation is 2.

**The limits to effectors in FSLH**

With the length induced contraction increase there is no associated decrease in the Hill coefficient and the shift of sensitivity to lower [Ca$^{2+}$] is positive but relatively small [2]. This is converse to the situation where the Ca$^{2+}$ restricting function of MyBP-C breaks down, as occurs in hypertrophic cardiomyopathy [6], with mostly mutations of MyBP-C. Similar loss of its function is found on inhibition MyBP-C binding to the heavy chain by a specific fragment of its binding domain reported by Kampourakis [12] or on reversible removal of MyBP-C by Hofmann [13].

As there is no change in the Ca$^{2+}$ cooperativity, Hill coefficient, the length induced shift to lower [Ca$^{2+}$] activation is simply the result of raising the effective Ca$^{2+}$ affinity of either the cTn-C or the MgADP-phosphomyosin [5,6,8,9]. A recent study by Zhang, et al. [14], with structural sensitive probes indicates that it is changes in the myosin head region, myosin light chain 2 (MLC2), that are responsible for the stretch induced amplification of maximal tension. Thus it is not the thin filament cTn-C Ca$^{2+}$ affinity but the increase in MgADP-phosphomyosin affinity for Ca$^{2+}$ that is key to length induced activation. They also report that minimal changes in the thin filament structure occur giving a very small increase in Ca$^{2+}$ sensitivity.

**The involvement of kinases**

de Tombe [15] and Akella [16] point out that the presence of both cTn-I and cTn-C together is a requisite for cardiac length induced activation, cTn-I is possibly a substrate for MLCK action. In FSLH cTn-I is not a substrate [1-4], for common PKA or PKC. The role of the antiparallel association of cTn-I and cTn-C has been investigated [3] in adrenergic stimulation, with PKA, but not the cTn-I phosphorylation on the threonine at residue 144. Only the threonine at position 144 in cardiac TnI is essential for length induced increase in contraction. Phosphorylation of the tail end serines is not involved in FSLH. This was shown by Tachampa, et al. [17] by substituting a hybrid skeletal Tn-I, with or without the serines, with added threonine at residue 144. Furthermore PKA phosphorylation of the tail end serines [3] reduces Ca$^{2+}$ sensitivity. Walker, et al. [18], using protein kinase C-βII (PKC-βII), an atypical PKC, have shown that phosphorylation of cTn-I on threonine 144 does increase the Ca$^{2+}$ sensitivity of contraction. They also demonstrate that sensitivity to Ca$^{2+}$ on MLCK action with Ca/calmodulin is independent of cTn-I modification, possibly swamped by MLC2 phosphorylation increase with calmodulin. PKC-βII has been shown to bind to the thin filament [19] on activation by phorbol ester where it could phosphorylate the cTn-I. However no evidence of diglyceride level change has been shown on length increase of the myocyte. If PKC-βII is involved it most probably is: (a) in the sarcoplasm, (b) is constitutively active for cTn-I and (c) is hindered by the kinase inhibitor β-arrestin as is MLCK, vide infra.

It is extremely likely that the phosphorylation targets of MLCK are much altered when bound to calmodulin, as are those of PKC-βII, in vivo it is accompanied by a calmodulin binding protein of unknown function. Demonstration of direct, uncatalyzed by calmodulin, phosphorylation by MLCK is lacking but this kinase is constitutively active and must occur when not inhibited. In FSLH there is no change in the level of adenralin activation hence not Ca/Calmodulin in action.

**The involvement of angiotensin**

By the use of knock-out mice the misnamed “multifunctional” transducer protein β-Arrestin has been shown by Abraham, et al. [20], to be essential to produce the length induced tension increase and small shift towards the cTn-C Ca$^{2+}$ sensitivity through the angiotensin II type 1 receptor (AT1R). This is not via the G-protein phosphoinositide-Ca$^{2+}$ second messenger system, i.e. there is no change in diastolic or systolic [Ca$^{2+}$] involved [15]. Protein phosphorylation [15] does though appear to be strongly involved as it is generally agreed that it is central to strength of contraction. In the case of MyBP-C and its functional insistence on Mg$^{2+}$ – Ca$^{2+}$ exchange [6] one might expect the observed change in contraction on its PKA induced phosphorylation [21] but not in FSLH. Kinase action on the tropolons does also modulate contraction strength [15], but again coupling with length induced changes has not been directly demonstrated and common PKA or PKC are not involved [1-4]. Downstream β-Arrestin stimulated Ca$^{2+}$-independent phosphorylation has also been muted [20] but again not shown.

The one key observation by Abraham, et al. [20] is that Losartan, a drug that blocks AT1R activation, is found to block the length induced enhancement of cardiac sensitivity. So on length increase it would seem the AT1R is activated by an unknown mechanism, reflected in the losartan action. In any striated muscle when AT1R is activated the next step in the sequence is phosphorylation of the activated AT1R tail by adrenergic receptor kinase (GRK) [22,23], not PKA or PKC. This phosphorylation is followed by translocation of β-Arrestin from the cytoplasm to bind strongly to the phosphorylated AT1R at the plasma membrane (PM). This binding inhibits further phosphorylation of the AT1R. β-Arrestin is a kinase inhibitor. This is from where its name
The binding of β-Arrestin to the angiotensin receptor, AT1R

Recently there has been interest in this interaction. In their review Turu, et al. [28], indicate that besides the well-known G protein pathways AT1R also activates a parallel signalling pathway through β-arrestins. This β-arrestin mediated activation affects the phosphorylation of cytoplasmic proteins such as mitogen-activated protein kinase (MAPK). Gagnon, et al. [29] have approached the mechanism of interaction of the β-arrestins with the AT1R via mapping the interacting moieties of the ligand and receptor by cross bridging reactions. They site specifically introduced the unnatural amino acid (UAA) p-azido-L-phenylalanine (azF) into the intracellular loops (ICLs) and the C-tail of AT1R. UV-mediated photolysis of 25 different azF-labeled AT1Rs cross-linked β-arrestin to angiotensin activated AT1R. As expected from the strength of the binding they demonstrated the presence of numerous different interactions of the β-arrestin with the receptor.

The effect of β-Arrestin translocation in muscle cells

Myosin light chain-2 (MYL2) and its phosphorylation [24,25,27,30,31] are central to the control of muscle contraction strength. MLCK activity would fit very well with the report by Zhang, et al. [14] of structural changes in the myosin heads on stretch activation. Generally in striated muscle MLCK is Ca2+ and calmodulin dependent but is also constitutively active, i.e. it has been found to have a variable fractions of its maximal activity in the absence of Ca2+ and calmodulin [21,24,25]. The variability probably arises with the level of self-phosphorylation reported. MLCK possibly has different substrate sensitivities when bound to Ca/ calmodulin or not. It is associated in vivo with a calmodulin inhibitor of unknown function that possibly inhibits low level Ca2+/Calmodulin activation thus preserving the suggested specificity of action. In the presence of Calmodulin it is specific for MYL2. Phosphorylation by MLCK of cTn-I has not been investigated. In the absence of AT1R activation MLCK is almost constitutively active, i.e. it has been found to have a variable fractions of its maximal activity in the absence of Ca2+/Ca2+ activation or deactivation is evident [1-4]. The indications are that the small length induced increase of skeletal muscle contraction is down to MLC2 phosphorylation alone and the more pronounced in cardiac muscle down to the added concerted action of the threonine phosphorylation on residue 144 of cTn-I.

What is excluded in FSLH

As with the specific essential phosphorylations above the amplitude of tension has been shown by de tombe, et al. [33], to be dependent on phosphorylation by PKA of both MyBP-C and cTn-I. Similar results were found with PKA phosphorylation of MyBP-C by Steltzer, et al. [34]. However, all or part of their results could also arise through the above mechanism by direct phosphorylation of the inactive AT1R by the cyclic AMP stimulated PKA addition. Distinguishing one kinase activation in the presence of all is not a trivial matter, however on stretch no evidence for common PKA or Ca2+ activation or deactivation is evident [1-4]. The functions and role of MYL2 and its regulation in general muscle function are well reviewed [32,35,36], albeit various isoforms of the enzymes involved are present depending on type and muscle location.

The future

Clearly much work investigating the mechanism of length induced angiotensin receptor activation is needed. We also want to know if the specificity of the kinases mentioned here, MLCK and PKC-βII are altered by Ca2+/calmodulin and autophosphorylation as is PKC-βII. The indications are that the small length induced increase of skeletal muscle contraction is down to MLC2 phosphorylation alone and the more pronounced in cardiac muscle down to the added concerted action of the threonine phosphorylation on residue 144 of cTn-I.

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