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

Gerry A. Smith, Department of Biochemistry University of Cambridge,
Tennis Court Rd. Cambridge CB2 1QW UK

gas1000@cam.ac.uk

Frank-Starling; angiotensin receptor; muscle contraction; myosin light chain kinase; Protein Kinase C-βII; myosin light chain phosphatase; β-arrestin; MgATP ⇔ MgADP-phosphomyosin; myosin LC affinity for Ca^{2+}

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 Ca^{2+}/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. Ca^{2+}/calmodulin is not activated in Frank-Starling, i.e. there are no diastolic or systolic [Ca^{2+}] changes. I suggest here that that the regulated inhibition is by myosin light chain phosphatase and/or β-arrestin. Angiotensin activation is by translocation of the β-arrestin from the sarcoplasm to the PM thus reducing its inhibition in the sarcoplasm, 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), 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 suggest that sarcomere length dependence of calcium sensitivity and the effects of phosphorylation by protein kinase A (PKA) of troponin-I (cTn-I) and C-protein are independent[1], and Steinberg[2] reports that protein kinase C (PKC) activation via Ca^{2+}/calmodulin would reduce contractile strength and no changes in lipid based activators are reported on stretch. Solaro[3] et at report that PKA phosphorylation has a negative effect on

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Ca^{2+} sensitivity but they get mixed up with the weak binding of Mg^{2+} to the troponins which has absolutely no effect on the activation of the cross-bridge. Solaro[4] also reports minimal effects of mimics of PKC phosphorylation of cTn-I. Neither of the common PKA or PKC take part in FSLH. My recent study[5] of the mechanism of FSLH led me to reconsider the Ca^{2+} cooperativity of contraction of normal heart[6] and its inhibition[7] by Mg^{2+}. The conclusion of this was that the cross-bridge is a doubly Ca^{2+} dependent ATPase that is restricted by myosin binding protein-C (MyBP-C) to CaATP as apparent substrate, i.e. the myosin bound Mg^{2+} has to be exchanged for Ca^{2+} for the cross-bridge to function in normal muscle. The consequential rate limiting step is release of phosphate which indicates Pi is initially reversibly transferred to the myosin light chain[8,9], MgATP ⇔ MgADP- phosphomyosin, rather than released directly. The exchange of Ca^{2+} for Mg^{2+}, along with the cross-bridge initiation by Ca^{2+} binding to troponin-C (Tn-C) releasing the Tn-I inhibition[10,11], makes the Ca^{2+} activation of the cross-bridge cooperative[5] i.e. the Hill coefficient for Ca^{2+} is 2. 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[5]. 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 or its deactivation/removal[6,12,13]. 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 myosin bound MgATP ⇔ MgADP- phosphomyosin[5,6, 8,9]. A recent study by Irving et al with structural sensitive probes[14] indicates that it is not the thin filament cTn-C Ca^{2+} affinity but the MgATP ⇔ MgADP-phosphomyosin affinity for Ca^{2+} increase that is key to length induced activation, in that changes in the myosin head region, myosin light chain 2 (MLC2), are responsible for the stretch induced amplification of maximal tension. They also report minimal changes in the thin filament structure occur giving a very small increase in Ca^{2+} sensitivity, possibly via threonine, residue 144, phosphorylation of cTn-I by myosin light chain kinase (MLCK) or PKC-βII, vide infra.

The involvement of angiotensin and kinases

The presence of both cTn-I[15] and cTn-C[16] together is a requisite for cardiac length induced activation, cTn-I is possibly a substrate for MLCK action in FSLH, but in FSLH is not a substrate[1,2,3,4] 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[17], the serines are not functional and PKA phosphorylation of these[3] reduces Ca^{2+} sensitivity. This is shown by substituting a hybrid skeletal Tn-I, with or without the serines, with added threonine at residue 144. Walker[18] et al using PKC-βII, an atypical PKC,

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have shown that phosphorylation of cTn-I on threonine 144 does increase the Ca^{2+} sensitivity, but also demonstrate that sensitivity to Ca^{2+} on myosin light chain kinase (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 change has been shown on length increase of the myocyte. If PKC-βII is involved it most probably is in the sarcoplasm, has to be constitutively active for cTn-I and be 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, 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, vide infra. In FSLH there is no change in the level of adrenalin activation hence not Ca/Calmodulin action.

By the use of knock-out mice the misnamed (personal interpretation) "multifunctional" transducer protein β-Arrestin[20] has also been shown 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), but 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 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[23] but not in FSLH. Although kinase action on the troponins 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[1,2,3,4]. Downstream β-Arrestin stimulated Ca^{2+}-independent phosphorylation has also been muted[20] but again not shown (does not exist? See later).

The one key observation is that Losartan, a drug that blocks AT1R activation, is found to block the length induced enhancement of cardiac sensitivity[20]. 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), not PKA or C, followed by translocation of β-Arrestin from the cytoplasm to bind strongly to the phosphorylated AT1R[20,24,25] 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 originates with discovery in the optic system[26,27]. Like a tiger no

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way does it change its spots with its location (see above “does not exist” and “misnamed”).

The effect of β-Arrestin translocation in muscle cells

Myosin light chain-2 (MYL2) and its phosphorylation[21,22,27,28,29] are central to the control of muscle contraction strength. MLCK activity would fit very well with the structural changes reported in the myosin heads on stretch activation, see above[14]. Generally in striated muscle MLCK is Ca^{2+} and calmodulin dependent but is also constitutively active, i.e. it has a variable fraction of its maximal activity in the absence of Ca^{2+} and calmodulin[21,22,23]. The variability probably arises with the level of self phosphorylation reported. MLCK possibly has different substrate sensitivities when bound to Ca/calmodulin or not and is associated in vivo with a calmodulin inhibitor of unknown function that possibly inhibits low level Ca^{2+}/Calmodulin activation thus preserving the suggested specificity of action. In the presence of Calmodulin it is specific for MYL2 and cTn-I has not been investigated. In the absence of AT1R activation MLCK is almost certainly partially inhibited by the kinase inhibitor β-arrestin that is normally present in the sarcoplasm, possibly binding the MYL2 bound MgATP ⇔ MgADP-phosphomyosin[2,6,8,9]. One can envisage a MYL2 phosphorylation level maintained by MLCK and light chain phosphatase[30] (LCP) and also regulated by β-arrestin, a central point of control of contraction. Reduction of β-arrestin inhibition of MLCK occurs on translocation of the β-arrestin to strongly bind to the AT1R on the PM on angiotensin activation. In length induced contraction strength the essential phosphorylation of cardiac cTn-I on threonine 144 possibly also occurs this way, clearly not by normal PKA or PKC[1,2,3,4]. This does not preclude direct PKA or PKC related changes in striated muscle when changes other than angiotensin activation occur such as adrenergic stimulation. This increase in phosphorylation of MYL2 by MLCK increases the affinity of the myosin bound ATP ⇔ ADP-Pi for Ca^{2+} over that for Mg^{2+}, more Ca^{2+} 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[15]. The myosin bound MgATP ⇔ MgADP-phosphomyosin equilibrium[8,9] which will be pH dependent and will probably have a bearing in the inhibitory binding of β-arrestin to the (MYL2)[8,9], appears phosphorylated, and indicates a possible control mechanism coupled with MYL2 phosphorylation.

As with the specific essential phosphorylations above the amplitude of tension has been shown by de tombe et al[31] 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[32]. However 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 C activation or deactivation is evident[1,2,3,4].

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The functions and role of MYL2 and its regulation in general muscle function are well reviewed[30,33,34], albeit various isoforms of the enzymes involved are present depending on type and muscle location.

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 Ca^{2+}/calmodulin and autophosphorylation as is PKC-βII. The indications are that the small length induced increase of skeletal muscle contraction is down to MLCK alone and the more pronounced in cardiac muscle down to the presence and phosphorylation of cTn-I.

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