Troponin I, Stunning, Hypertrophy, and Failure of the Heart

R. John Solaro

Two articles in this issue of Circulation Research point to the significance of the loss of cardiac troponin I (cTnI) from the cell, the altered structure of cTnI, and the altered function and interactions of cTnI as possible key events in hypertrophy and ischemia/reperfusion injury. These studies also provide strong evidence for the hypothesis of Kusuoka and Marban,1 who first suggested that stunning is associated with reversible breakdown and replacement of damaged myofilament proteins. Later work from Marban’s laboratory4 identified cTnI proteolysis as the cause of the reversible cardiac dysfunction in stunning. The results reported by McDonough et al1 extend this and other observations,5,6 correlating altered thin-filament regulation with dysfunction resulting from cardiac stunning and more severe episodes of ischemia with and without reperfusion. In agreement with an earlier report5 suggesting that the proteolysis of cTnI occurred between residues 188 and 199, McDonough et al1 demonstrate that with moderate ischemia/reperfusion, cTnI is degraded at its C-terminal end by removal of 17 amino acids generating cTnI1–188. With longer durations of ischemia and ischemia/reperfusion, there is also an N-terminal truncation generating 2 other degraded forms of the protein (cTnI63–193 and cTnI73–193). What is also new and potentially important is evidence that cTnI1–193 forms a covalent complex with the N-terminus of cTnC, a region that contains the single regulatory Ca2+-binding domain, and with the C-terminal residues of TnT that are important in transducing the Ca2+ binding signal to tropomyosin (Tm). McDonough et al1 propose that this complex formation occurs through the action of transglutaminase.

Also in this issue, Huang et al2 report the first experiments generating a mutant mouse in which the cTnI gene has been ablated by gene targeting. The mice are normal at birth and in their first 2 weeks of life. However, as expression of slow skeletal TnI (ssTnI), the fetal form, wanes and stops, the mice hearts demonstrate diastolic dysfunction and reduced myofilament responsiveness to Ca2+.; by about day 18, they die of heart failure. These data show clearly that depletion of TnI in the myofilaments leads to severe cardiac abnormality and that expression of the fetal ssTnI isoform does not persist even when the life of the cells is threatened by the absence of the adult cardiac isoform.

Why Would We Expect Loss, Truncations, and Aberrant Cross-Linking of cTnI To Be Functionally Significant?

From all that we know concerning the complexity of myofilament control mechanisms and the role of cTnI in contraction and relaxation (see Solaro and Rarick7 for review), it is not surprising that a mouse cannot live without cTnI and that alterations in cTnI affect myofilament function. cTnI occupies a central position at the crossroads of the signaling pathways between Ca2+ binding to cTnC and crossbridge binding to actin. An N-terminal region of cTnI anchors cTnC to the thin filament by a strong interaction with its C-terminus. When Ca2+ is not bound to the regulatory site on cTnC, a C-terminal region of cTnI and a highly basic inhibitory region (Ip) tether cTnI to actin. When Ca2+ is bound to the cTnC regulatory site, these regions of cTnI move away from actin and bind to the N-terminus of cTnC. As shown by the data of Huang et al1 and by in vitro experiments,8 removal of cTnI from myofilaments induces activation of the myofilaments independent of the Ca2+ concentration. Current concepts7 indicate that cTnI through its interactions with actin and cTnT establishes relaxation by promoting a “closed state” in which crossbridges react with the thin filament, but in a weak non–force-generating reaction. cTnI may also participate in a “blocked state” in which crossbridges are physically hindered from reacting with the thin filament. Along with tropomyosin and cTnT, cTnI, which appears to be an elongated protein extending over nearly 2 actin monomers, could sterically block myosin crossbridges.

Loss and/or truncation of cTnI at either end of the molecule would be expected to alter the mechanism by which myofilaments achieve the diastolic state and respond to Ca2+. Can truncation of cTnI by 17 C-terminal amino acids account for the previous functional data, demonstrating a fall in maximum force-generating ability and a change in Ca2+ sensitivity in stunned rat myocardium?3–6 Studies8 with in vitro preparations reconstituted with recombinant cTnI missing C-terminal amino acids suggest that this loss of 19–20 amino acids could cause this effect. Removal of 11 amino acids from the C-terminus had no apparent effects on the function of mouse heart cTnI. However, cTnI1–188, which is missing only 5 more amino acids than cTnI1–193, had impaired ability to induce the relaxed state and to produce the fully activated state. Moreover, a mutant cTnI missing 60 residues (cTnI 1–151) was unable to support Ca2+-dependent activation when reconstituted into the myofilaments. Thus, amino acids 152–199

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comprise a C-terminal region of cTnI that is essential for Ca²⁺-dependent control of myofilament activity.⁸

McDonough et al⁸ also report that severe ischemia/reperfusion injury is associated with a proteolysis of N-terminal amino acids of cTnI. We would expect that truncation of cTnI at it N-terminus would result in severe abnormalities in myofilament control mechanisms. This region of cTnI reacts with the C-terminus of cTnC, anchoring it to the thin filament during relaxation and contraction.⁹ This region of cTnI also binds to the C-terminus of cTnT, an interaction important in signal flow to Tn.⁷ Myofilament preparations reconstituted with cTnI₁₄–₈₁ and cTnI₁₀–₂₁₁ show a severe loss of Ca²⁺ activation, although relaxation is near normal, presumably owing to the interaction of the C-terminus and Ip of cTnI with actin.⁹

cTnI is not only essential for Ca²⁺-dependent activation of the cardiac myofilaments, but it is also an important control element during intrinsic and extrinsic control of cardiac function. Thus, proteolysis or loss of cTnI would be expected to interfere with this mode of cellular regulation. The myofilaments are able to sense extrinsic sympathetic and parasympathetic control mechanisms largely through phosphorylation of cTnI at protein kinase A (PKA) sites in the unique N-terminal extension and at protein kinase C (PKC) sites near the N-terminus and in the Ip.⁷ Phosphorylation of cTnI at the PKA sites depresses the ability of Ca²⁺ to activate the myofilaments by a reduction in the affinity of cTnC for Ca²⁺. Evidence is accumulating that this effect of cTnI phosphorylation is an important aspect of the relaxation effect of adrenergic stimulation.⁷ Phosphorylation at the PKC sites appears to reduce the affinity of crossbridges for the thin filament.¹⁰ Moreover, both PKA phosphorylation of the myofilaments, most likely at cTnI,¹¹ and isoform switching from cTnI to ssTnI¹² have been reported to alter length-dependent Ca²⁺ activation of the myofilaments. Length-dependent activation is well accepted to be a main cellular mechanism for Starling’s law. Thus, in addition to disrupting the essential function of cTnI in Ca²⁺ control of myofilament activation, loss or proteolysis of cTnI may compromise modulation of myofilament response to Ca²⁺. Certainly, proteolysis of 62 amino acids from the N-terminus of cTnI, as reported by McDonough et al.,¹ would remove the serine residues at positions 22 and 23 that are substrates for PKA as well as the serine residues at positions 45 and 45 that are substrates for PKC. It is not clear, although possible, that truncation at the C-terminus of cTnI has global effects on protein structure that alter cTnI as a substrate for PKA and PKC. It is also unclear whether myofilaments containing cTnI₁₄–₈₁ or other truncated forms of cTnI retain the same length dependence of activation as native myofilaments.

Could Loss or Altered Structure and Function of cTnI Actually Trigger the Hypertrophic Process?

One of the most exciting aspects of the area of research covered in the articles in this issue is the prospect that proteolysis of cTnI may be an initial or triggering insult engaging the hypertrophic process. Perhaps the strongest evidence that a primary defect in the myofilaments could lead to hypertrophy is the now extensive evidence that missense mutations in sarcomeric proteins are genetically linked to familial hypertrophic cardiomyopathy (FHC). Indeed, mutations in the C-terminus of cTnI have been genetically linked to FHC.¹³ Clearly, it would be of interest to compare the alterations in cellular mechanical and Ca²⁺ dynamics induced by the presence of cTnI₁₄–₈₁ in the myofilaments with those associated with FHC or stunning.

In any case, a pressing question is this: How do transcriptional and translational mechanisms, which induce hypertrophy, decode a signal arising from a mutant or altered protein in the myofilaments? In the case of proteolysis or loss of cTnI, a plausible set of circumstances is that an initial insult would lead to depressed diastolic and systolic function, resulting in sustained elevations of end-diastolic pressure and volume as well as altered amplitude and dynamics of [Ca²⁺] transients. Associated changes in heart rate could also affect the frequency of the Ca²⁺ transients. Alterations in cellular Ca²⁺ dynamics and stretch of the myocardium are 2 prominent mechanisms by which hypertrophy could evolve. I think that the studies¹⁴ demonstrating differential activation of transcription by variations in Ca²⁺ dynamics are especially compelling here and that the role of an altered myofilament response to Ca²⁺ has been generally ignored as an important element in control of these Ca²⁺ dynamics. The potentially significant roles of altered Ca²⁺ dynamics and calcineurin in the evolution of hypertrophy are under current intense investigation.¹⁵ Stretch is known to release hypertrophic factors such as endothelin and angiotensin. These agonists engage the PKC pathway, resulting in elaboration of phosphorylation cascades, inotropic effects, and alkalosis. My earlier speculation¹⁶ that depression of myofilament activity associated with phosphorylation of cTnI may be important in PKC-related hypertrophy/failure has been demonstrated in a transgenic mouse model overexpressing PKC β.¹⁷ Interestingly, this mechanism may also be modified if cTnI is broken down by proteolysis.

Do Changes in cTnI Signaling in Animal Models Have any Relevance to Human Conditions?

Whether or not the changes documented in the studies by Huang et al² and McDonough et al.¹⁸ are important in human ischemia/reperfusion injury, hypertrophy and failure are far from clear. To my knowledge, there is no published evidence for the presence of a breakdown product such as cTnI₁₄–₈₁ in human hearts. Moreover, studies⁶ with postischemic pig myocardium did not demonstrate this breakdown product, although they did confirm that myofilament response to Ca²⁺ is depressed. However, when partially exchanged into skeletal myofilaments, the troponin regulatory complex from the stunned, but not the control myocardium, was able to induce a desensitization to Ca²⁺. An important aspect of this study is that only a small percentage of the Tn needed to be replaced to induce desensitization. Similarly, results have been obtained in recent studies¹⁸ with transgenic mice overexpressing a mutant form of cTnT linked to FHC. Substitution of only 5% of the native cTnT with the mutant form could induce altered response to Ca²⁺. Although it is possible that antibodies used in the studies with pig heart myocardium were not...
able to detect loss of C-terminal amino acids, the results point to the possibility of other defects. For example, oxidation of the Tn complex could be an important factor. This possibility is suggested from the data of Perez et al., showing that xanthine oxidase inhibitors, which are known to blunt the dysfunction of stunning, sensitize myofilaments to Ca\(^{2+}\).

Thus, careful examination of preparations from human hearts with appropriate antibodies for detection of breakdown products appears to be required to test the hypothesis that cTnI breakdown is important for generation of cardiac stunning.

As pointed out by Huang et al., there is no evidence that cTnI is lost from myofilaments of cells in human heart failure. Moreover, there is no evidence ssTnI is reexpressed in human hearts in end-stage heart failure. Whether the changes in cTnI or ssTnI content or structure have special relevance to pediatric cardiology has not been investigated.

As with all studies aimed at understanding the human pathology, there are problems with sampling, adequate controls, and sample preservation. Clearly, though, the work presented in these 2 studies in this issue increases our understanding of the potential molecular mechanisms of cellular injury and provides a new and valuable model for investigating structure/function of cTnI in myofilament relaxation and contraction. These studies also indicate that to test the hypothesis that cTnI breakdown is associated with human cardiac dysfunction requires the understanding that antibodies with appropriate epitopes be used and that the possibility exists that small changes in the myofilament proteins may lead to big changes in function.

References


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