Regulation of cardiac contractile function by troponin I phosphorylation

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Abstract

Cardiac troponin I (cTnI) is a key regulatory protein in cardiac muscle contraction and relaxation, linking Ca\(^{2+}\)–troponin C binding with activation of crossbridge reactions with the thin filament. In recent years, it has become increasingly apparent that myofilament properties as well as changes in intracellular Ca\(^{2+}\) have a major role in the dynamic modulation of contractile function. The phosphorylation of specific serine and threonine residues on cTnI by several different kinases represents a major physiological mechanism for alteration of myofilament properties. Furthermore, altered thin filament function plays an important role in the contractile dysfunction associated with heart failure. Modification of cTnI by protein kinases A and C has been extensively studied with especially useful information deriving from (a) in vitro studies in reconstituted detergent-skinned fibre bundles in which endogenous cTnI was replaced with various targeted cTnI mutants and (b) transgenic animals in which endogenous cTnI was similarly manipulated through overexpression of cardiomyocyte-targeted cTnI mutants. cTnI may also be specifically modified by protein kinase G, p21-activated kinases and by dephosphorylation. This review focuses on recent advances in understanding the mechanisms of cTnI modification by these kinases and the consequent functional effects both under physiological conditions and in pathophysiological settings.

Keywords: Myocardial contractility; Troponin I; Phosphorylation; Protein kinases

1. Introduction

Troponin I (TnI) is the ‘inhibitory’ unit of the troponin complex associated with the thin filament, and inhibits actomyosin interactions at diastolic levels of intracellular Ca\(^{2+}\). Binding of Ca\(^{2+}\) to troponin C (TnC) during systole induces conformational changes that relieve the inhibitory influence of cardiac TnI (cTnI), thereby promoting actomyosin crossbridge formation and contraction [1]. Upon decline of cytosolic [Ca\(^{2+}\)] to diastolic levels, Ca\(^{2+}\) dissociates from TnC and restores the inhibitory action of cTnI on actomyosin interaction. As such, cTnI represents a key regulatory protein in cardiac muscle contraction and relaxation, linking Ca\(^{2+}\)–TnC binding with activation of crossbridge reactions with the thin filament.

Alterations in contractile function may result either from changes in the size or duration of the Ca\(^{2+}\) transient and/or changes in myofilament response to Ca\(^{2+}\). While the pivotal role of Ca\(^{2+}\) in regulating contraction and relaxation is well recognized, it has become increasingly apparent that myofilament properties also have a major role in the dynamic modulation of contractile function [1,2]. For example, crossbridge kinetics rather than the rate of Ca\(^{2+}\) decline may represent the rate-limiting step of relaxation at physiological temperature and frequency, at least in some species, e.g., rat [3]. Recent evidence also suggests that altered thin filament function plays an important role in the contractile dysfunction associated with human heart failure [4].

Phosphorylation of specific serine and threonine residues on cTnI by several different kinases represents a major physiological mechanism for alteration of myofilament properties [5]. Changes in cTnI phosphorylation status have
been reported in failing human hearts [6–8] and may reflect changes in the balance between kinase and phosphatase activities. The use of specific in vitro and in vivo molecular approaches has significantly advanced our understanding of the role of cTnI phosphorylation. This review deals with the functional significance of cTnI phosphorylation under physiological and pathophysiological conditions. Biophysical and structural aspects of cTnI regulation and the role of other myofilament proteins have been reviewed recently [1,2,5] and are not addressed here.

2. Physiological roles of cTnI phosphorylation

2.1. Protein kinase A (PKA)-mediated phosphorylation

β-Adrenergic stimulation is a major physiological mechanism to meet increases in circulatory demand, acting through positive inotropic and lusitropic effects. These actions involve PKA-mediated phosphorylation of several proteins, including sarcolemmal L-type Ca$^{2+}$ channels, phospholamban, sarcoplasmic reticulum (SR) ryanodine receptor channels, cTnI and myosin binding protein (MyBP-C). Increased L-type Ca$^{2+}$ current and SR Ca$^{2+}$ cycling (secondary to phospholamban phosphorylation, removal of its inhibitory effect on SR Ca$^{2+}$ uptake, and an increase in SR Ca$^{2+}$ load) are thought to be largely responsible for the positive inotropic effect of β-stimulation. PKA-dependent phospholamban phosphorylation also significantly contributes to the lusitropic effects of β-stimulation, by increasing SR Ca$^{2+}$ reuptake. In recent years, a significant contribution of PKA-dependent cTnI phosphorylation to both the lusitropic and positive inotropic effects of β-stimulation has become evident.

PKA phosphorylation of cTnI reduces myofilament Ca$^{2+}$ sensitivity and shifts the force–pCa relationship rightwards, an effect that involves phosphorylation of serine residues 22 and 23 (Ser22/23 in the mouse and Ser23/24 in human, excluding the initiating methionine) in the N-terminal (reviewed in [1,2,5]). These residues are part of a 27–33 amino acid N-terminal domain that is unique to cTnI and absent in the slow skeletal isoform (ssTnI). PKA-induced cardiac myofilament densitization to Ca$^{2+}$ and an increase in the rate of Ca$^{2+}$ dissociation from TnC may contribute to an acceleration of relaxation. In addition, PKA-dependent cTnI phosphorylation increases crossbridge cycling rate and enhances unloaded shortening velocity [9–15], which may also contribute to β-agonist-induced lusitropy. These effects on crossbridge dynamics have, however, been somewhat controversial since some studies found no change in crossbridge cycling rate or shortening velocity following β-stimulation [16–19]. The reasons for these differences are not entirely clear but experimental factors may contribute.

The use of transgenic mice with specific perturbations of cTnI expression has been invaluable in definitively establishing the contribution of PKA phosphorylation of cTnI to the lusitropic effects of β-stimulation. Fentzke et al. [13] generated a transgenic mouse in which cTnI in cardiomyocytes is stoichiometrically replaced by ssTnI, which lacks the N-terminal domain of cTnI containing the Ser residues normally phosphorylated by PKA. In this model, β-agonist-induced lusitropy was markedly blunted in isolated myocytes, isolated hearts, and in vivo [13,20–22]. Transgenic replacement with ssTnI also abolished PKA-induced increases in unloaded shortening velocity [13] and crossbridge cycling rate [14], suggesting that cTnI phosphorylation in the N-terminal is essential for these effects. Pi et al. [23,24] generated a transgenic mouse with cardiac expression of a mutated cTnI in which PKA phosphorylation sites are replaced by non-phosphorylatable alanines. In myocytes harbouring these phosphorylation-site mutants, the reduction in myofilament Ca$^{2+}$ sensitivity following β-stimulation was abolished [24] and its lusitropic effect markedly reduced [23]. In an alternative approach, Takimoto et al. [25] generated transgenic mice overexpressing mutated cTnI with replacement of Ser22/23 by aspartate residues to mimic constitutive phosphorylation. These mice demonstrated enhanced left ventricular (LV) relaxation in vivo compared to non-transgenic animals, especially at high heart rates, a difference that was normalized in the presence of isoproterenol. An increased effect of cTnI phosphorylation on relaxation rates at higher frequency has also been reported by Tong et al. [26].

Recent findings suggest that the contribution of PKA-mediated cTnI phosphorylation to the relaxant effects of β-stimulation vary according to loading conditions and may be especially important during aoxutonic contraction, i.e., loaded shortening as occurs in vivo during LV ejection [20,25,27,28]. Notably, transgenic mice with “pseudo-phosphorylated” cTnI PKA sites had much less afterload-induced delay in relaxation than non-transgenics, a difference abolished by isoproterenol [25]. These results suggest that cTnI phosphorylation at PKA sites plays a critical role in enhancing relaxation during increases in afterload, which serves to limit afterload-induced delay in relaxation.

While the above data indicate a significant role for cTnI phosphorylation in the lusitropic effects of β-stimulation, an important question has been its contribution relative to that of phospholamban phosphorylation. Studies utilizing phospholamban knockout mice generated contradictory results, with initial findings that phospholamban ablation abolishes the relaxant effect of isoproterenol [29] followed by reports that β-agonist-induced lusitropy is independent of phospholamban phosphorylation [20,27,30]. Such discrepancies could at least partly be due to differences in loading conditions, which may be important for myofilament-based relaxant effects as discussed above [20,27,28]. However, recent studies in which phospholamban knockout animals were crossed with non-phosphorylatable cTnI transgenics have provided definitive evidence that both phospholamban phosphorylation and cTnI phosphorylation are necessary for the full lusitropic response to β-agonists [20,23].
PKA-induced cTnI phosphorylation also appears to contribute significantly to β-agonist-induced positive inotropy, despite the fact that it reduces myofilament Ca\(^{2+}\) sensitivity and shifts the force–pCa relationship rightward. This apparently counterintuitive effect may be accounted for by the fact that PKA phosphorylation increases crossbridge cycling rate and shortening velocity \([9–15]\)—which may be especially important during loaded shortening or auxotonic contraction. Thus, PKA treatment of skinned myocytes (in which the contributions of sarcolemmal Ca\(^{2+}\) channels and phospholamban are absent) increased absolute peak power during loaded shortening, at least in part by speeding loaded crossbridge cycling rates \([15]\). Similarly, a myofilament-based effect was proposed to explain the \(\beta\)-mediated increase in network and power output in cardiac muscles performing work-loop contractions during SR inhibition \([28]\). Consistent with this idea, we recently reported that the positive inotropic response to \(\beta\)-stimulation is severely blunted in the hearts of ssTnI transgenic mice studied in the ejecting mode \([22]\). A striking finding in this study was that the blunting of positive inotropic responses in ssTnI mice was most evident in ejecting hearts (particularly with respect to ejection phase indices) whereas inhibition of isoproterenol effects was much less apparent in isovolumic hearts or unloaded cardiomyocytes \([22]\). Further support for a contribution of PKA phosphorylation of cTnI to positive inotropy comes from Takimoto et al. \([25]\), who found that transgenic mice with pseudophosphorylated cTnI PKA sites exhibited significantly enhanced systolic function at baseline and a greater frequency-dependent enhancement of systolic function in vivo. The latter study provides the first suggestion of an important role for cTnI phosphorylation (as well as the SR) in the positive force–frequency relationship of normal myocardium. It should be noted that the above results have been obtained in rodent preparations and confirmation in other species including human myocardium is required.

Taken together, the above data indicate that PKA-mediated cTnI phosphorylation likely contributes to enhancement of systolic function as well as relaxation during \(\beta\)-stimulation, complementing the effects of increased Ca\(^{2+}\) transient amplitude and accelerated Ca\(^{2+}\) transient decline in bringing about increased positive inotropy and lusitropy under conditions of heightened cardiac demand (Fig. 1).

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**Fig. 1.** Schematic showing the major pathways involved in the physiological regulation of cardiac function through cTnI phosphorylation. \(\varphi\) represents phosphorylation by the kinase indicated (left panel), leading to changes in myofilament properties and altering overall muscle or heart function as indicated on the right. Dephosphorylation by protein phosphatase 2A would be expected to reverse the effects of phosphorylation. The contractile effects of cTnI phosphorylation are determined by the combination of kinases activated, the specific sites phosphorylated and the activities of protein phosphatases—on the background of the other effects induced by the specific agonist in question (e.g., the increase in Ca\(^{2+}\) transient amplitude and alteration in kinetics that occur with \(\beta\)-adrenergic stimulation). NH\(_2\) and COOH indicate the amino and carboxy termini of cTnI, respectively. Ang II=angiotensin II; ET-1=endothelin-1. Other abbreviations as used in the text.
2.2. Phosphorylation by protein kinase C (PKC)

cTnI is a substrate for phosphorylation by the PKC kinases, which may be activated by G-protein-coupled receptor agonists such as angiotensin II, endothelin-1 and the α-adrenergic agonist phenylephrine. Since PKC activation in intact cells may have multiple myofilament-based and non-myofilament effects, it is not surprising that negative [31,32] or positive inotropic effects [23,33] and negative [23,31] or positive lusitropic effects [34] have all been reported. Furthermore, there may be PKC isoform-specific effects; PKCα, PKCβ and PKCγ are expressed in the normal adult heart of most species, with expression of PKCα and PKCβ1/2 increasing during hypertrophy and heart failure [4,31,35]. The specific PKC isoforms responsible for the effects discussed below remain poorly defined, with suggestions that PKCα may be especially important [36,37] but evidence also of a role for PKCβ2 in heart failure [31].

PKC may phosphorylate cTnI at Ser22/23, Ser43/45 and Thr144 [38]. The best information on the functional effects of phosphorylation at these sites comes from either (1) in vitro studies in reconstituted detergent-skinned fibre bundles in which endogenous cTnI was replaced with various targeted cTnI mutants, or (2) transgenic animals in which endogenous cTnI was similarly manipulated through the overexpression of cardiomyocyte-targeted cTnI mutants. Transgenic mice facilitate analyses of contractile function in intact (including in vivo) as well as skinned preparations but may be complicated by secondary changes in properties of cTnI and/or other proteins such as TnT [36,39,40]. The acute nature of genetic modification in reconstituted in vitro preparations reduces these problems; however, functional analyses are usually limited to skinned muscle or sliding filament assays.

Using reconstituted preparations, phosphorylation at Ser43/45 (achieved either with PKCα or by mutating to glutamic acid to mimic constitutive phosphorylation) decreased maximum Ca2+-activated tension in skinned fibres and maximal sliding velocity in in vitro motility assays, probably by decreasing crossbridge detachment rate [37,39,41]. Interestingly, Burkart et al. [37] also found that phosphorylation at Ser43/45 caused a decrease in Ca2+ sensitivity of force production whereas a reduction in the Ca2+ sensitivity of filament sliding required Thr144 phosphorylation. These results suggest that phosphorylation of different sites on cTnI may have distinct effects in regulating tension versus filament sliding velocity. (It should be noted that in a study in which myofibrillar preparations were prepared from hearts of transgenic or wild-type mice that had been perfused with phorbol ester or endothelin-1 [24], Ser43/45 phosphorylation was associated with an increase in Ca2+ sensitivity and a reduction in maximal Mg-ATPase rate.) The effects of PKC-mediated cTnI phosphorylation at Ser43/45 with regard to whole heart or muscle contractile function remain poorly understood. A decreased maximal Ca2+-activated tension and crossbridge cycling rate [37,39,41] would be predicted to reduce contractility and prolong relaxation. Accordingly, in transgenic mice in which 50% of native cTnI was replaced by a mutant cTnI with Ser43/45 sites substituted by non-phosphorylatable alanines, the effect of phenylephrine to reduce tension in papillary muscles was significantly blunted [32]. Furthermore, in detergent-extracted fibre bundles from these hearts, the negative effects of PKC activation on maximum tension were abolished [39]. In a separate transgenic mouse with replacement of all five PKC phosphorylation sites by alanines, the effect of endothelin-1 to delay twitch relaxation in intact cardiomyocytes was abolished whereas positive inotropic responses were unaffected [23]. These data suggest that PKC-mediated cTnI phosphorylation plays an important role in prolonging twitch duration, probably by slowing crossbridge kinetics [23,37]. This effect likely involves Ser43/45 phosphorylation since phosphorylation of Ser23/24 leads to faster twitch relaxation [13,20,22,23,34]. PKC-mediated cTnI phosphorylation may also affect the economy of force development. In studies on fibre bundles from wild-type mice and transgenic mice with partial replacement of native cTnI by mutant TnI lacking phosphorylatable Ser43/45, Pyle et al. [39] reported that PKC-mediated changes in tension (e.g., with phenylephrine or endothelin-1) were associated with an increased tension cost in the transgenic group. Such a difference could account for the finding that these transgenic hearts are more prone to development of ischaemic contracture in vitro [42]. Pi et al. [24] also proposed that PKC-induced reduction in maximum Mg-ATPase rate may promote more efficient ATP utilisation by slowing crossbridge turnover, which could improve cardiac efficiency. These data are in line with earlier work suggesting beneficial effects of endothelin-1 on cardiac efficiency [43].

PKC can also cross-phosphorylate the PKA sites at Ser23/24 [41], resulting in reduced myofilament Ca2+ sensitivity and changes opposite to those of Ser43/45 phosphorylation in terms of crossbridge cycling rate. PKCβ2 (like PKA) may also reduce myofilament Ca2+ sensitivity, based on studies in a transgenic mouse overexpressing this isoform in the heart [31], but whether this involves Ser23/24 phosphorylation has not been reported. Interestingly, recent data examining global cardiac function in vivo found that in transgenic mice with Ser43/45 mutated to alanine there was increased cTnI phosphorylation at Ser23/24, which resulted in enhanced contraction and relaxation under basal conditions [36]. These data suggest that “basal” contractile function may reflect a balance between effects resulting from PKC-dependent Ser43/45 phosphorylation (which reduces systolic function and delays relaxation) and PKA-dependent Ser23/24 phosphorylation (which has positive inotropic and lusitropic effects) [36]. In this regard, PKA and PKC might also have opposing effects on cardiac efficiency.

Although the precise effects of PKC-dependent cTnI phosphorylation remain to be fully established, an emerging
consensus from the data presented above would be that Ser43/45 phosphorylation reduces maximal Ca\(^{2+}\)-activated tension and in concert with phosphorylation of Thr144 reduces sliding velocity, and that these effects may be relatively beneficial in energetic terms at least in the non-diseased heart (Fig. 1). Furthermore, there may be significant interdependence between PKC- and PKA-mediated effects at Ser43/45 and Ser23/24, respectively.

2.3. Phosphorylation by cyclic GMP-dependent protein kinase (PKG)

PKG exists as 2 isoforms, PKG I and PKG II [44]. PKG I is expressed at relatively low levels in cardiomyocytes [44,45] and can phosphorylate cTnI at Ser23/24, although at an ~100-fold slower rate than PKA [44,46]. However, Thr1T may serve as an anchoring protein for PKG, thus facilitating preferential and rapid cTnI phosphorylation [44]. The cGMP–PKG pathway in cardiomyocytes is activated by various autocrine/paracrine agents, including nitric oxide (NO) synthesized by coronary microvascular endothelial cells or cardiomyocytes (reviewed in [47]) and the natriuretic peptides—i.e., atrial natriuretic peptide (ANP) synthesized by atrial tissue, brain natriuretic peptide (BNP) synthesized by ventricular tissue and C-type natriuretic peptide (CNP) synthesized by endothelial cells [48,49]. Peroxynitrite (formed from reaction between NO and superoxide) may also activate the cGMP–PKG pathway [50].

PKG-induced cTnI phosphorylation at Ser23/24 should have similar effects to those of PKA (Fig. 1). Indeed, treatment of skinned cardiac preparations with PKG reduces myofilament Ca\(^{2+}\) sensitivity [51], with a greater effect in failing compared to non-failing human myocardium [52]. However, the effects of PKG on crossbridge kinetics and shortening velocity have yet to be determined. The contribution of cTnI phosphorylation by PKG to overall changes in contractile function has been difficult to ascertain, especially since PKG may have other effects such as reduction of the L-type Ca\(^{2+}\) current or increased SR Ca\(^{2+}\) uptake [47]. Furthermore, agents such as NO that raise cGMP may have additional non-cGMP-dependent effects, while cGMP itself can influence the activity of several phosphodiesterases and thereby modulate function by altering cAMP levels [47]. Nevertheless, one fairly consistent effect of NO donors, PKG activators or agonists that release NO in a variety of preparations and species including humans is an acceleration of myocardial relaxation and/or a reduction in diastolic tone [53–57]. Thus, NO has been suggested to play an important role in regulating diastolic tone and ventricular filling probably through PKG-dependent pathways [47]. In intact cardiomyocytes, these effects of NO or PKG activators resulted from a reduction in myofilament Ca\(^{2+}\) sensitivity [55–57], and were blocked by pharmacological inhibition of PKG [55–58] or by knockout of PKG I in a transgenic model [59]. Furthermore, in isolated rat hearts, treatment with an NO donor caused an increase in cTnI phosphorylation at Ser23/24 [57]. It has been suggested that PKG could reduce myofilament Ca\(^{2+}\) sensitivity by inducing an intracellular acidosis [60], but we found that the negative inotropic and relaxant effects of NO donors were independent of intracellular acidosis [55,57]. Interestingly, the cardiodepressant effects of peroxynitrite have also been largely attributed to reduced myofilament Ca\(^{2+}\) responsiveness, at least partly mediated by PKG [50].

Of the natriuretic peptides, CNP has effects comparable to NO, i.e., a transient positive inotropic effect followed by a negative inotropic effect and sustained acceleration of relaxation [45,48,49]. These effects were enhanced in transgenic mice with cardiac-specific overexpression of PKG I [45]. The contractile effects of CNP have been associated with increased cTnI phosphorylation [48] although there may also be simultaneous PKG-dependent phospholamban phosphorylation [45,48,49] and acceleration of Ca\(^{2+}\) transient decline [45].

2.4. Dephosphorylation by protein phosphatases
cTnI phosphorylation is influenced by protein phosphatases as well as kinases but the mechanisms regulating cTnI dephosphorylation remain unclear. The major phosphatase thought to dephosphorylate cTnI and phospholamban is type 2A protein phosphatase (PP2A) [61]. Activation of PP2A and ensuing dephosphorylation of regulatory proteins is involved in the anti-adrenergic effects of adenosine and muscarinic receptor activation [62]. Adenosine A1 receptor activation with N\(^6\)-cyclopentyladenosine (CPA) attenuated \(\beta\)-adrenergic agonist-induced positive inotropic effects and was associated with decreased cTnI and phospholamban phosphorylation [62]; these effects were blocked by PP2A inhibition [62]. CPA also reduced maximum unloaded shortening velocity of skinned rat cells under either baseline conditions or following \(\beta\)-stimulation, suggesting a potential role for the adenosine–PP2A pathway in inhibition of crossbridge cycling rates [63]. However, CPA had no effect on myofilament Ca\(^{2+}\) sensitivity in these experiments [63].

The signalling pathway linking adenosine A1 receptor stimulation to PP2A activation may be complex, and is suggested to involve G\(_i\)-coupled stimulation of guanylyl cyclase followed by cGMP-dependent activation of p38 mitogen-activated protein kinase (MAPK) and subsequent translocation and activation of PP2A [64]. In this regard, recent studies indicate that p38MAPK may acutely modulate cardiac contractile function in addition to its chronic effects on cell hypertrophy and apoptosis [65]. In isolated rat cardiomyocytes, p38MAPK inhibition with a pharmacological inhibitor SB203580 rapidly increased contractile amplitude whereas transfection with the constitutively active upstream activator MKK3Be led to reduction in contractile amplitude, an effect abolished by a dominant negative p38MAPK mutant [65]. Likewise, in skinned rat myocytes, p38MAPK activation induced by exposure to
arsenite caused a reduction in maximal force production [66]. The effects of p38MAPK in intact myocytes were unrelated to alterations in the Ca\textsuperscript{2+} transient and were therefore attributed to a reduction in myofilament response to Ca\textsuperscript{2+} [65]. However, p38MAPK was unable to phosphorylate purified cTnl in vitro, and definitive evidence that p38MAPK acts by influencing cTnl phosphorylation either directly or indirectly (e.g., via PP2A) remains lacking.

2.5. Phosphorylation by p21-activated kinases

Recent data suggest that cTnl may also be a potential target for another family of serine/threonine protein kinases, the p21-activated kinases (Pak) of which at least 3 isoforms (Pak1, Pak2 and Pak3) exist in the heart [67]. Paks are regulated by the small G proteins, Cdc42 and Rac1, and are implicated in the development of cardiac hypertrophy and heart failure [67]. In vitro addition of Pak3 to skinned rat cardiac fibres increased myofilament Ca\textsuperscript{2+} sensitivity with no change in maximal Ca\textsuperscript{2+}-activated force [67]. These effects were associated with Pak3-induced phosphorylation of myofilament proteins, including cTnl which was phosphorylated at a novel site, Ser149, located in the region forming a Ca\textsuperscript{2+}-sensitive interaction with the N-terminal regulatory domain of TnC [67]. In a separate study, constitutive activation of Pak1 by cardiac myocyte gene transfer also increased myofilament Ca\textsuperscript{2+} sensitivity, but in this case Pak1 appeared to act by inducing PP2A-dependent dephosphorylation of cTnl and MyBP-C [61]. Whether different Pak isoforms have both direct phosphorylating and indirect (via PP2A) dephosphorylating effects on distinct parts of the cTnl molecule remains to be established as does the significance of Pak-induced changes in cTnl phosphorylation in the dynamic regulation of cardiac contractility (Fig. 1).

3. Alterations in cTnl phosphorylation in pathological states

3.1. PKA-dependent cTnl phosphorylation in cardiac hypertrophy and failure

The contractile dysfunction that occurs in chronic left ventricular hypertrophy and heart failure has largely been attributed to alterations in Ca\textsuperscript{2+} handling that result mainly from alterations in the expression and properties of Ca\textsuperscript{2+} regulatory proteins such as the SR Ca\textsuperscript{2+} ATPase and the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (for review, see [68]). However, increasing evidence suggests that alterations in myofilament properties may also play a significant role both in experimental models and in humans. Among the changes reported in hypertrophied and failing human myocardium are a reduction in maximal myofibrillar ATPase activity [69] and maximal Ca\textsuperscript{2+}-activated force [4,70]; a decrease in shortening velocity and crossbridge cycling rates [71–73]; and an increased economy of contraction [74]. Alterations in myofilament proteins including myosin heavy and light chains [8] and cTnl [4,6–8,52] may be involved. In failing rat heart, alterations in cTnT have also been found [75].

Several studies have reported a significant reduction in basal cTnl-phosphorylation at PKA sites (Ser23/24) in human heart failure [6–8] although this is not a universal finding [73,76]. These changes may be due to down-regulation of the β-adrenergic signalling pathway (e.g., reduced β-adrenoceptor density, increased expression of G\textsubscript{i} proteins [7,8,70]) and/or upregulation of PP2A activity [76,77], both of which may be altered in heart failure. A reduction in basal PKA-dependent cTnl phosphorylation may increase myofilament Ca\textsuperscript{2+} sensitivity and impair crossbridge cycling (as frequently observed in failing hearts), thereby contributing to impaired relaxation and reduced power output. The increased basal Ca\textsuperscript{2+} sensitivity may also account for the observation that PKA activation induces greater reduction in myofilament Ca\textsuperscript{2+} sensitivity [8,52,78] in failing compared to non-failing myocardium.

3.2. PKC-dependent modulation of cTnl function in hypertrophy and failure

PKC isoform expression profile alters during heart failure, with increased expression and activity of PKC\textbeta [4,35]. An increase in PKC-dependent phosphorylation of cTnl and cTnT has been suggested to contribute to the reduction in maximum Ca\textsuperscript{2+}-activated force and increase in Ca\textsuperscript{2+} sensitivity found in failing human myocardium [4]. The role of PKC\textbeta\texttwo has been addressed using transgenic mice overexpressing PKC\textbeta\texttwo in heart [31,79–81]. PKC\textbeta\texttwo overexpression was associated with significant cardiac hypertrophy and fibrosis, as well as systolic and diastolic dysfunction [79]. A significant component of contractile dysfunction in this model was intrinsic to the cardiac myocyte, in which cell shortening and relengthening velocities were severely impaired probably as a result of a reduction in myofilament responsiveness to Ca\textsuperscript{2+} [31]. However, in a different transgenic model with a more modest increase in PKC\textbeta expression, the phenotype was one of mild progressive hypertrophy associated with diastolic dysfunction [80]. In the latter model, isolated cardiomyocytes showed enhanced contractility resulting from an increased Ca\textsuperscript{2+} transient [81]. However, skinned fibres from transgenic hearts demonstrated a significant reduction in maximum Ca\textsuperscript{2+}-activated force, attributed to increased cTnl phosphorylation. Thus, the depressed myofilament function apparent in skinned fibres was compensated for in intact myocytes by an increased Ca\textsuperscript{2+} transient [81]. These data suggest that the contractile effects of PKC upregulation may be dependent upon the stage of hypertrophy; in compensated hypertrophy, the effects of PKC on Ca\textsuperscript{2+} transients may outweigh its effects to depress myofilament function whereas the latter may predominate in more advanced hypertrophy and heart failure [81]—Fig. 2.
Interestingly, transgenic overexpression of PKCε is also associated with a decrease in maximum Ca²⁺-activated force in conjunction with increased phosphorylation of both cTnI and cTnT. However, in this model, the decreased contractility was most likely the result of changes in myosin heavy and light chain isoform expression and associated alterations in crossbridge function [82]. It would therefore appear that while increased cTnI phosphorylation by PKC and consequent depression of myofilament function may contribute to contractile defects in hypertrophy and heart failure, additional effects of PKC on intracellular Ca²⁺ and other myofilament proteins also play a major role.

### 3.3. Role of cTnI phosphorylation in sepsis

Severe gram-negative bacterial infection may result in hypotension and multiple organ dysfunction, with cardiac dysfunction being a major feature of the pathophysiology (reviewed in [83]). Severe endotoxaemia induces an intrinsic impairment of cardiac contractility, evident in isolated perfused hearts [84,85], cardiac muscle [84,86] and single cardiomyocytes [87,88] from septic animals. In some studies, impaired contractility has been associated with changes in Ca²⁺ handling [87]. However, many studies report depressed contractility in the absence of a reduced Ca²⁺ transient [88,89], suggesting alterations in myofilament properties. Cardiac tissue from septic animals was found to exhibit reduced myofilament Ca²⁺ responsiveness [84,86,88,90] and decreased myofibrillar Mg²⁺-ATPase activity [91].

Alterations in cardiac myofilament properties in sepsis may be directly related to the phosphorylation status of cTnI, MyBP-C and/or myosin light chain-2 [88,90,91]. An increase in cTnI phosphorylation at Ser 23/24 was suggested as a potential mechanism underlying reduction in myofilament Ca²⁺ sensitivity in systemic sepsis [88–90]—Fig. 2. In support of such a mechanism, endotoxaemic rat hearts showed increased cTnI phosphorylation at Ser 23/24 compared with controls [88]. To assess if the relationship between increased cTnI phosphorylation at these sites and decreased contractility in endotoxaemia is causal, we recently investigated the contractile effects of experimental endotoxaemia in transgenic mice with replacement of cTnI by ssTnI [92]. These studies showed that ssTnI hearts were significantly protected against endotoxaemia-induced contractile dysfunction, most probably by preserving myofilament Ca²⁺ responsiveness by preventing TnI phosphorylation at PKA-sensitive sites. However, additional mechanisms are also likely to contribute to contractile dysfunction in this setting. Changes in myofilament function in endotoxaemia probably represent the net effect of changes in phosphorylation status [91] and degradation [87] of various contractile proteins (e.g., cTnI, myosin light chains, MyBP-C) following the activation of intracellular signalling cascades and kinases.
4. Conclusions

Modulation of myofilament properties by alterations in cTnI phosphorylation has profound effects on cardiac contractility and pump function. The effects of cTnI phosphorylation depend upon the combination of kinases activated, the specific sites phosphorylated, and the activities of protein phosphatases. Phosphorylation at Ser 23/24 (e.g., by PKA or PKG) results in reduction in myofilament Ca\(^{2+}\) sensitivity and an increase in crossbridge cycling rate, leading to acceleration of relaxation and an increase in power output but a reduced economy of contraction. Conversely, phosphorylation at Ser 43/45 (by PKC) is associated with reduced maximum Ca\(^{2+}\)-activated contraction. Conversely, phosphorylation at Ser 43/45 (by PKC) is associated with reduced maximum Ca\(^{2+}\)-activated contraction. Conversely, phosphorylation at Ser 43/45 (by PKC) is associated with reduced maximum Ca\(^{2+}\)-activated contraction.

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