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Tear me down: Role of calpain in the development of cardiac ventricular hypertrophy

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Abstract

Cardiac hypertrophy develops most commonly in response to hypertension and is an independent risk factor for the development of heart failure. The mechanisms by which cardiac hypertrophy may be reversed to reduce this risk have not been fully determined to the point where mechanism-specific therapies have been developed. Recently, proteases in the calpain family have been implicated in regulating the development of cardiac hypertrophy in preclinical animal models. In this review, we summarize the molecular mechanisms by which calpain inhibition has been shown to modulate the development of cardiac (specifically ventricular) hypertrophy. The context within which calpain inhibition might be developed for therapeutic intervention of cardiac hypertrophy is then discussed.

Keywords

cardiac hypertrophy; calpain; calpastatin; NF-κB inhibition; HSP90; β3 integrins

Introduction

Cardiac hypertrophy develops most commonly in response to hypertension and is an independent risk factor for the development of heart failure and more generally an increased morbidity and mortality[1]. Although the mechanisms by which cardiac hypertrophy may be reversed to reduce the increased risk have not been fully determined to the point where mechanism-specific therapies have been developed, epidemiologic studies suggest that regression of hypertrophy is a salutary clinical goal [2, 3]. The increase in cardiomyocyte mass involves the increase in protein synthesis stimulated by a variety of intracellular signaling pathways [4]. In parallel, changes in the rate of protein degradation occur, both increasing and decreasing depending on the hypertrophic stimuli [5, 67-10]. Therefore, the reversal of cardiac hypertrophy therapeutically would likely involve either decreasing protein synthesis and/or increasing the rate of protein degradation. In this review, we discuss the newly discovered role that the calpain proteolytic system plays in mediating signal transduction pathways involved in cardiac ventricular hypertrophy.

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Degradation of proteins in the cardiomyocyte, as in other cells, involves 3 parallel systems that function both separately and cooperatively: 1) the ubiquitin proteasome system; 2) lysosomes and the process of autophagy; and 3) the calpain proteases. The ubiquitin proteasome system (UPS) includes a series of enzymes that target specific substrate proteins for degradation by the 26S proteasome. The UPS-mediated regulation of cardiac mass has been shown to be mediated by multiple ubiquitin ligases, the components of the UPS that give it its specificity, as well as the proteasome. The ubiquitin ligases muscle ring finger-1 (MuRF1) and MAFbx (aka atrogin-1) play a role in regulating cardiac mass [11-14]. There is some evidence suggesting that inhibition of the proteasome may play a role in regulating cardiac hypertrophy in vivo, at least experimentally [15]. However, there is also evidence that proteasomal inhibition actually causes cardiac hypertrophy under baseline conditions and enhances the development of hypertrophy in aortic-banded animals [16], leaving the issue unclear as to whether inhibition of the proteasome in the setting of cardiac hypertrophy is protective or detrimental.

The second system involved in cardiac protein degradation involves lysosomal proteolysis. Inhibiting lysosome function in the heart results in an approximately 25-30% reduction in the overall rate of protein degradation [17]. While lysosome activity does not appear to affect myosin degradation, it does play a role in the degradation of organellar proteins, including mitochondrial cytochromes and microtubules [17, 18]. Autophagy, which is involved with targeted lysosomal degradation of proteins and organelles, occurs constitutively at a low level during normal cardiac function [19]. However, during times of cardiac stress, autophagic activity increases, presumably as an adaptive response to the significant amount of structural remodeling that accompanies the cardiac stress response [20-22].

The third proteolytic system active in the heart is the calpain system, which includes a family of calcium-dependent, non-lysosomal cysteine proteases that are expressed ubiquitously within all cells and whose function in muscle appears to involve both atrophic and hypertrophic pathways [23, 24]. Several recent publications have reported the role of calpain proteases in regulating the development of cardiac hypertrophy. These studies add numerous novel details to our understanding of how calpains, and their interactions with specific cell signaling pathways, might be involved in the complex regulation of cardiac hypertrophy. With few therapies available to regulate or reverse cardiac hypertrophy, the identification of cardiac calpains as a potential therapeutic target is exciting since a large body of work already exists describing the regulatory pathways involved in this form of proteolysis. This review gives a brief background on the role of calpains in the heart and then focuses on their role in regulating cell signaling in cardiac hypertrophy.

The calcium ion-dependent papain-like protease (Calpain) family of proteases

Members of the calpain family of intracellular Ca^{2+}-activated proteases are critical mediators of the action of calcium. At least 16 calpains have been described, most found ubiquitously, although some being tissue specific (see recent review by Bukowska et al., 2010 [25]). Calpains are generally localized to the cytosol as inactive pro-enzymes that may be activated by increases in intracellular calcium. Calpains operate by processing proteins, through interactions with a limited number of motifs, to transform their activities and structure. Calpain activity is specific and does not induce widespread degradation of proteins (see Table 1). The conventional calpains, calpain 1 and 2 (also known as μ and m-calpain), are tightly regulated by an endogenous inhibitor, called calpastatin[23]. The four inhibitory domains of calpastatin bind reversibly to the active calpain domains to inhibit their activity. The activity of calpain is also inhibited by post-translational modification by phosphate groups[23]. For example, phosphorylation of Ser369 by protein kinase A (PKA) prevents the

formation of the active site necessary for calpain activity [26]. Calpains have been implicated in degrading a diverse array of substrates, involved in various areas of biology (see Table 1).

Calpains in the heart in health and disease

Both calpain 1 and calpain 2 are present in moderate amounts within the muscle where they are localized to the Z disk of muscle fibers [27, 28]and have been associated with the in vitro degradation of sarcomeric proteins such as α-tropomyosin [29, 30]. The majority of the studies looking at calpain activity in the heart have focused on the role of this proteolytic system in response to pathological cardiac conditions, such as post-ischemic cardiac injury [31-33]. However, at least one study has examined the role of the calpains in baseline cardiac function. In cultured cardiomyocytes, calpain 1, but not calpain 2, is found to be active at physiological levels of calcium, resulting in the proteolysis of specific substrates (e.g. desmin and protein kinase Cα) as well as increased protein ubiquitination and protein turnover by the 26S proteasome [34]. Mice in which the calpain inhibitor calpastatin is ectopically expressed at increased levels in the heart exhibit a decrease in ubiquitination of some specific cardiac proteins, but no overall change in cardiac protein ubiquitination, suggesting that the effect of calpain 1 (the only calpain moiety affected by the overexpression of calpastatin) is on the actual ubiquitination step and not on 26S proteasome activity[34]. Most interestingly, however, is the finding that inhibition of calpain 1 activity by forced expression of calpastatin results in a progressive, dilated cardiomyopathy that is accompanied by an accumulation of aggregated protein complexes, formation of autophagasomes, and destruction of sarcomere integrity [34]. Together, these findings suggest that calpain 1 activity is essential for normal cardiac function and is integral to the regulation of protein turnover of specific cardiac proteins (the identity of which have not yet been confirmed) whose accumulation leads to disruption of normal myofibril activity and subsequent cardiomyopathy. More broadly, calpains have been implicated in cell cycle (calpain 2), contraction, apoptosis (calpain 2), cell migration (calpain 1), cell differentiation (calpain 2) and cellular signal transduction in muscle (calpain 3) [35-40].

The involvement of calpain activity in the progression of cardiac pathologies is well known [41]. Calpain activity mediates alterations in sarcomere structure and affects contractile dysfunction in ischemia reperfusion injury (calpains 1 and 2), myocardial stunning (calpain 1) and atrial fibrillation (calpain 1) [25, 32, 42-47]. One mechanism by which calpain 1 activity may be linked to atrial fibrillation is through its cleavage of specific L-type Ca^{2+} channel proteins, leading to the disruption of the excitation-contraction coupling mediated by Ca^{2+} channels [48-50]. Likewise, calpain activation in reperfusion following ischemic insult has been proposed as one possible mechanism mediating myocyte cell death, either by activation of apoptosis via Bid and/or necrosis by increasing fragility due to degradation of sarcomeric proteins (recently reviewed by Inserte et al., 2009 [51]). In isolated rabbit hearts exposed to global ischemic injury, calpain activity cleaves Bid, resulting in the enhanced release of cytochrome c from mitochondria leading to apoptosis [52]. There is also evidence for calpain involvement in congestive heart failure as well as in the atrophic remodeling that accompanies cardiac unloading. Ventricular tissue isolated from congestive heart failure patients exhibits a marked increase in calpain expression [53]. In milder cases of congestive heart failure (rated as class II on the New York Heart Association (NYHA) scale) an increase in the protein level of calpain 1, but not calpain 2 is observed. However, when heart failure progresses to a more severe level (NYHA III and IV) a significant increase in the protein levels of both calpains is seen. Mechanical unloading of the failing human heart results in a slight increase in calpain 1 expression and a significant increase in expression of calpain 2 [54]. Likewise, in the unloaded rat heart, both calpain 1 and 2 expression and activity levels are increased [54], providing further evidence of calpain's involvement in the tissue remodeling associated with various cardiac pathologies.

Calpain regulation of signaling in cardiac hypertrophy

The development of pathologic cardiac hypertrophy, such as that induced by pressure overload, occurs in response to the stimulation of multiple signaling pathways that in turn activate a handful of transcription factors to activate pro-hypertrophic gene expression programs (see recent reviews[4, 55, 56]). Despite the complexity of these signaling pathways, only a relatively few number of transcription factors have been shown to drive this process, including NF-κB, GATA4, NFAT, SRF, and MEF2 [55-59]. The signaling pathways driven by these transcription factors facilitate hypertrophic growth of cardiomyocytes and activate so-called "fetal genes". The concept of re-expressing genes normally expressed only during the fetal period of heart development is well established during the development of cardiac hypertrophy [4, 55]. Briefly, the activation of transcription factors such as SRF and GATA4 induce specific gene expression and protein synthesis globally. In this respect, a number of influential signaling pathways have been identified as important mediators of cardiomyocyte hypertrophy. These pathways include the angiotensin II-induced NF-κB /NFAT pathway, the Akt signaling pathway, and the stretch-induced (β3 integrin-mediated) signaling pathways [4]. Interestingly, recent studies have implicated calpain in regulating cardiac hypertrophy by its specific interaction through each of these signaling pathways.

Blocking calpain activity disrupts cardiac hypertrophy by inhibiting NF-κB activation

Angiotensin II (Ang II), a key component of the rennin-angiotensin-aldosterone system, induces cardiomyocyte hypertrophy by interacting with the angiotensin II type I receptor, a G protein coupled receptor. Chronic infusion of Ang II in mice results in the development of hypertension and cardiac hypertrophy [60]. In parallel, increases in calpain activity and decreases in calpastatin (the endogenous inhibitor of calpain), expression are induced. In transgenic mice that constitutively express calpastatin, the chronic infusion of Ang II fails to induce cardiac hypertrophy, although these mice do still develop hypertension. Both Ang II and calpain 1 signaling activate the NF-κB [61-63] and calcineurin/NFAT signaling pathways [64, 65]. Infusion of Ang II leads to a robust increase in expression of the p65 subunit of NF-κB in the nuclei of cardiomyocytes (indicating enhanced activity), an effect that is considerably blunted in calpastatin transgenic mice [60]. Surprisingly, Ang II infusion induces equal amounts of NFAT activation in calpastatin transgenic mice and wild-type mice. Together, these results suggest that calpain 1 activity mediates Ang II-induced cardiac hypertrophy via a NFAT-independent, NF-κB-dependent pathway [60].

The mechanism by which Ang II activates NF-κB has been elucidated in recent studies published by Heidrich, et al., 2008[66]. They have identified that Ang II induces calcium release after binding to the Ang II receptor via the inositol 1,4,5-triphosphate receptor ($InsP_3R$) pathway in cardiomyocytes [66]. They found that the $InsP_3R$-dependent release of calcium, which turns on chromogranin B (CGB), leads to NF-κB activation and expression of brain natriuretic peptide, a protein whose expression is increased in cardiac hypertrophy and heart failure. It has been postulated that calpains may mediate chromogranin activation in response to increased calcium in this system[67]. NF-κB activation may be related to chromogranin B activation as well. The evidence for this comes from studies which demonstrate an attenuated NF-κB activity in cardiomyocytes with reduced chromogranin B expression [66]. The proposed relationship of these signaling pathways is summarized in Figure 1 (highlighted in red). This figure describes the relationship between the increased Ang II, a hormone increased in most patients developing cardiac hypertrophy [68], which then activates calpain activity, resulting in enhanced downstream NF-κB activity to induce the "pro-hypertrophic" genes in cardiomyocytes.

β-adrenergic stimulation of calpain activity blocks eNOS and Akt signaling

In addition to calpain 1's role in activating cardiac hypertrophy by activating NF-κB, calpain activity can also lead to the inhibition of pro-hypertrophic signaling pathways. During the development of cardiac hypertrophy in humans, β-adrenergic stimulation occurs in parallel to stimulation by other G protein coupled receptors, like the Ang II receptor [69]. β-adrenergic stimulation has recently been shown to activate calpains and block eNOS activity and Akt signaling, both of which have been implicated as "pro-hypertrophic" signaling pathways [70]. Experimentally, cardiomyocyte stimulation with the β-adrenergic agonist isoproterenol increases calpain activity while decreasing the activity of calpastatin [71]. Cardiac hypertrophy induced by chronic isoproterenol administration in ovariectomized female rats leads to calpain-mediated breakdown of the sarcomere, as evidenced by a decrease in the calpain substrate sarcomeric proteins dystrophin, utrophin, and spectrin protein expression (see Figure 1, pathways in blue) [70]. In addition, a marked reduction in eNOS activity, a parallel decrease in HSP90 protein levels, and increase in caveolin 3 proteins levels were seen. Decreased Akt phosphorylation and increased glycogen synthase kinase 3β phosphorylation are also seen with chronic β-adrenergic stimulation. Although this study did not go so far as identifying the link between increased calpain and decreased eNOS and Akt activity, it is possible that HSP90 may be the commonality between these 2 effects, as described below.

The interaction of HSP90 with eNOS and Akt enhances their activity[72, 73]. Experimentally, calpain 2 degrades HSP90 in culture [74, 75]. Evidence for the link between calpain and decreased Akt and eNOS activity comes from experiments performed in endothelial cells. Calpain inhibition in pulmonary artery endothelial cells leads to increased eNOS activity and nitric oxide production[72], likely through the HSP90-mediated enhancement of eNOS activity [76, 77]. Similar to the effects on eNOS, the HSP90/Akt complex formation is critical to Akt activity/phosphorylation [76]. Calpain also inhibits Akt activity in diaphragmatic muscle by reducing HSP90 expression and decreasing Akt activity, an effect that coincides with reduced HSP90/Akt complex formation [73]. Finally, isoproterenol administration in rats decreases HSP90 and eNOS activity in the heart at the same time Akt signaling is inhibited [70]. Since both Akt-GATA4 and PKC activation are crucial to the development of cardiac hypertrophy experimentally [55], calpain's destabilization of HSP90 may be one mechanism which inhibits pro-hypertrophic signaling pathways in cardiomyocytes. The proposed mechanisms by which isoproterenol activates calpain to inhibit downstream eNOS and Akt signaling through its disruption of HSP90 is proposed in Figure 1 (highlighted in blue).

β3 integrins induce calpain activity to enhance cell survival and induce cardiac hypertrophy

In the previous sections, we've discussed how Ang II stimulates calpain activity to enhance pro-hypertrophic signaling pathways via NF-κB and how β-adrenergic stimulation induces calpain activity to decrease eNOS/Akt stimulation, possibly inhibiting hypertrophic signaling. In addition to these roles for calpain activity in the development of cardiac hypertrophy, recent studies have reported that β3 integrin-dependent calpain 1 activation inhibits apoptosis in cardiomyocytes which in turn leads to the development of cardiac hypertrophy. A major mechanism by which mechanical forces activate cardiac hypertrophy is through integrins [78]. Integrins are a class of receptors that extend through the plasma membrane and connect the intracellular sarcomere to the extracellular matrix [79]. These receptors are located at specific sites in the plasma membrane: the intercalated discs and costameres. These receptors detect mechanical stress and act as initiators of downstream signaling through a number of signaling pathways including focal adhesion kinase [79]. Recent studies have implicated integrin signaling in calpain activation and the development of cardiac hypertrophy. β3 integrin -/- mice subjected to trans-aortic constriction for four

weeks to induce pressure overload cardiac hypertrophy exhibit both an increase in cardiomyocyte cell death (by TUNEL assay) and a decrease in ventricular mass compared to wild type control mice [78]. Pressure overload in β3 integrin -/- mice also leads to an enrichment of calpain 1 in cardiac muscle [80], whereas pretreatment with calpeptin, a specific inhibitor of calpain, before pressure overload induction in β3 integrin -/- mice attenuates the enhanced cell death as determined by TUNEL staining [80]. Although the role of calpain1 in β3 integrin-mediated cardiac hypertrophy has not been definitively determined, it is possible that it serves a regulatory function to balance the processes of cell survival and cell death[80]. In cultured cardiomyocytes, β3 integrin stimulation induces both calpain activity and NF-κB (independent of calpain activation NF-κB). This in turn leads to NF-κB-mediated enhancement of expression of the pro-survival factor cIAP [78]. The absence of these pro-survival signals (and therefore the unabated pro-apoptotic influence of calpain activation) in the β3 integrin -/- mice may account for the enhanced cardiomyocyte apoptosis seen during pressure overload hypertrophy in these mice. Although much of this pathway still needs to be elucidated, these studies demonstrate a link between the mechanically-induced β3 integrins, calpain 1 activity, and the maintenance of cell survival, possibly involving the pro-hypertrophic cIAP and NF-κB signaling as summarized in Figure 1 (highlighted in green).

Calpains broadly consolidate stress signaling to induce cardiac hypertrophy

During the development of cardiac hypertrophy, calpain activities are enhanced by numerous stimuli, suggesting that calpain activation may represent a general mechanism by which the cell responds to external stress, including stress hormones (norepinephrine, AngII) and stretch (via β-integrins), as discussed above. Another way calpain activity influences cardiac hypertrophy by responding to external stress is by activation via reactive oxygen species. NADPH oxidases (NOXs) are membrane-bound enzymes found in the plasma membrane that function to generate superoxide by transferring electrons from NADPH to molecular oxygen to produce superoxide, a reactive free radical. Recent studies have shown that stimulating adult rat ventricular cardiomyocytes via norepinephrine increases NADPH oxidase (NOX) activity and reactive oxygen species (ROS) generation, leading to enhanced calpain 1 activation and apoptosis [81]. Inhibiting the predominant NOX in cardiomyocytes, $gp91^{phox}$-NADPH oxidase, using apocynin or diphenyleneiodonium, or inhibiting ROS using the antioxidant N-acetyl-cysteine protects cardiomyocytes from apoptosis at the same time as preventing the activation of calpain 1 [81]. Similarly, direct inhibition of calpain prevents cardiomyocyte apoptosis, presumably by blocking the norepinephrine-induced calpain activation that is mediated by NADPH-oxidase [81]. These studies indicate a central role of calpains that intersect with numerous diverse stress signaling pathways to activate cardiac hypertrophy (see Figure 1, orange).

The role of calpains in protein degradation in cardiac hypertrophy

Many of the calpain substrate proteins listed in Table 1 play an important role in cardiac function, raising the obvious question of what would calpain degradation of these proteins mean for cardiac health? For example, the ability of calpains to degrade focal adhesion kinase, calcineurin, and caspases is striking given the prominent role these proteins play in cardiac hypertrophy. Focal adhesion kinase is a broadly expressed tyrosine kinase that detects biomechanical stress and then signals to induce cardiac hypertrophy[79, 82] Subsequent calpain activation caused by this cardiac hypertrophy, could result in the degradation of focal adhesion kinase (or calcineurin, another purported calpain substrate) thereby explaining, in part, the inhibitory effect that calpain activation has on cardiac hypertrophy development [82, 83]. Alternatively, if increased calpain activity enhances the degradation of caspases in cardiac hypertrophy, protection against cell death and development of cardiac

hypertrophy might occur, confounding our understanding of how degradation of these reported calpain substrates might effect cardiac hypertrophy. In addition, many of the proteins listed in Table 1 (for example calcineurin, caspases, and G protein α subunit) were identified as calpain degradative targets in the brain [84][85, 86]. Therefore, with the notable exception of calpain-mediated degradation of caspases, dystrophin, utrophin, spectrin and the L-type Ca2+ channels[48-50] discussed in previous sections, the role of calpain degradation of known structural and signal transduction pathways in the heart has yet to be determined.

Calpastatin in cardiac health and disease

Although this review focuses mainly on the role that calpains play in the regulation of cardiac ventricular hypertrophy, a brief discussion on the role that the endogenous inhibitor of calpain, calpastatin, plays in physiological and pathological cardiac function is warranted. The regulation of calpastatin has been reported in experimental myocardial infarction and cardiac ischemia reperfusion injury (see Table 2)[87, 88]. In the left ventricular free wall, calpastatin protein levels are not affected days 1, 3, 7, and 14 after myocardial infarction in Wistar rats [87]. Other studies have identified that ischemia reperfusion injury causes a down-regulation of calpastatin activity. When hearts from Wistar rats are perfused ex vivo and challenged with a 20 minute global ischemia, followed by reperfusion for up to 30 minutes, calpastatin activity was reduced 40-60% when assayed for their ability to inhibit calpain 1 and calpain 2 [88]. Parallel decreases in protein levels of calpastatin were also identified after reperfusion (summarized in Table 2) [88].

A number of studies have been published detailing the effect of calpastatin overexpression, both systemically and specifically within the heart [34, 60, 89, 90]. Since the methods used and the parameters evaluated differed between the various studies, it is difficult to get a clear idea of the effect of overexpression of calpastatin on cardiac function. For example, when calpastatin is overexpressed in all tissues, the baseline cardiac functions (as determined by heart rate, heart work, and rate of contraction and relaxation) do not differ from wild type mice [89]. Likewise, no difference was seen between wild type and transgenic animals in relation to cardiac calpain activity (measured by the accumulation of 145/150-kDa spectrin BDP) or calpain 1 and calpain 2 expression [60]. In unloaded hearts of mice overexpressing calpastatin cardiomyocyte size also decreases, suggesting that other proteolytic systems may compensate for calpain activity [54]. However, when calpastatin is overexpressed specifically in the heart, a much different picture is seen. Mice in which cardiac calpastatin is increased such that myocardial calpain 1 activity is inhibited by 58% exhibit a slowly progressive dilated cardiomyopathy, illustrated by decreased ventricular ejection performance and responsiveness to ß-adrenergic stimulation [34]. In addition, approximately half of the transgenic mice evaluated display atrial arrhythmias. Despite the difference in baseline phenotype of the systemic and cardiac-specific calpastatin mice, there is a common finding of decreased cardiac pathology in both types of transgenic mice when the mice are challenged with pathological stimuli. Mice in which calpastatin is systemically overexpressed, exhibit a decrease in the development of Ang II-induced cardiac hypertrophy and subsequent cardiac dysfunction when compared to wild type mice [60]. Similarly, isolated rat hearts in which calpastatin is overexpressed (via adenoviral transfection) exhibit a significant decrease pathology associated with I/R injury, as evidenced by greater left ventricular functional recovery and a decrease in degraded cardiac troponin I levels (a target of calpain degradation)[90].

Calpain inhibition as a therapeutic tool to treat cardiac hypertrophy

To date, only a handful of studies have been published examining the potential of calpain inhibition as a therapeutic approach for treatment of ventricular hypertrophy. In a feline

model of right ventricular pressure overload, the calpain inhibitor calpeptin was administered intravenously both before and during the development of pressure overload [91]. Control animals exhibited numerous physiological and pathological changes following 24 hours of pressure overload, including an increase in calpain protein expression and activity, a decrease in calpastatin levels, an increase in caspase-3 activation and an increase in cellular markers of programmed cell death in cardiomyocytes. In contrast, the animals that had been treated with calpeptin did not develop any of these changes, strongly suggesting the involvement of the calpain system in these cellular responses to the pressure overload as well as demonstrating a promising effect of calpain inhibition in the whole animal. Likewise, anesthetized, open-chested pigs, treated with the calpain inhibitor MDL-28170 before the induction of right ventricular pressure overload, exhibited a significant degree of protection from the development of right ventricular wall dysfunction compared to animals that were not treated with the calpain inhibitor [92]. Lastly, rats treated with isoproterenol to induce ventricular hypertrophy exhibited a mild protection from hypertrophic changes when dosed with the cysteine protease inhibitor E-64c I hour prior to the treatment with isoproterenol, suggesting that calpain inhibition is effective in decreasing the effects of β-adrenergic-mediated cardiac hypertrophy [93]. Although these studies hint at the possible effectiveness of calpain inhibition in the development of ventricular hypertrophy, the safety and long-term effects of calpain inhibition remains to be determined.

Summary

The studies reviewed here largely demonstrate that inhibiting calpain activity during the induction of cardiac hypertrophy attenuates or prevents the development of hypertrophy, suggesting that calpains may be a novel target for treating cardiac hypertrophy. A number of issues remain to be answered, however, if calpain is to be developed as a therapeutic target. Most importantly, it needs to be determined if inhibiting calpain has any long-term side effects in the heart. The pre-clinical studies reported so far do not look at long term outcomes of animals in which calpain inhibition prevents cardiac hypertrophy. Secondly, it needs to be determined if inhibiting calpain activity in established pressure overload-induced cardiac hypertrophy can reverse it enough to reduce the associated progression to heart failure and/or reduce the associated morbidity and mortality. Lastly, how does calpain inhibition affect other organ systems in both animals and humans that would undoubtedly be affected by a systemic anti-calpain approach. These questions are of primary importance given the array of calpain substrates found in the heart that have obvious relevance to cardiac health and disease (see Table 1). If calpain inhibition proves to be a viable target for cardiac therapies, studies have shown that calpains have a number of chemical qualities which make theoretically good targets for which synthetic inhibitors can be developed from a medicinal chemistry point of view[94].

The recent studies described in this review demonstrate that calpain enzymes are emerging as unique entities within the protease systems active in the heart in that they appear to be able to respond to global stresses. As described above, calpains are capable of both activating and inhibiting signal transduction pathways involved in common hypertrophic responses to diverse external stimuli, including reactive oxygen species, stretch stimuli through β-integrins, and broadly through activation by G-protein coupled receptors such as Ang II and the β-adrenergic receptor (summarized in Figure 1). In addition, calpain activation mediates both pro- and anti-hypertrophic effects through NF-κB and eNOS/Akt signaling, respectively, although the contribution of each of these mechanisms in cardiac hypertrophy is not entirely worked out. Given the complexity of the multiple signal transduction pathways activated during cardiac hypertrophy, there are likely other pathways affected by calpain activation that have not been determined.

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Non-standard abbreviations

Akt	serine/threonine protein kinase
Ang II	angiotensin II
cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanosine monophosphate
eNOS	endothelial nitric oxide synthase
GSK3β	glycogen synthase kinase 3 beta
InsP3R	inositol 1,4,5-triphosphate receptor
NOX	NADPH-oxidases
PKA	protein kinase A
PKC	protein kinase C
NFAT	Nuclear factor of activated T cells
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
ROS	reactive oxygen species
TAC	trans-aortic constriction

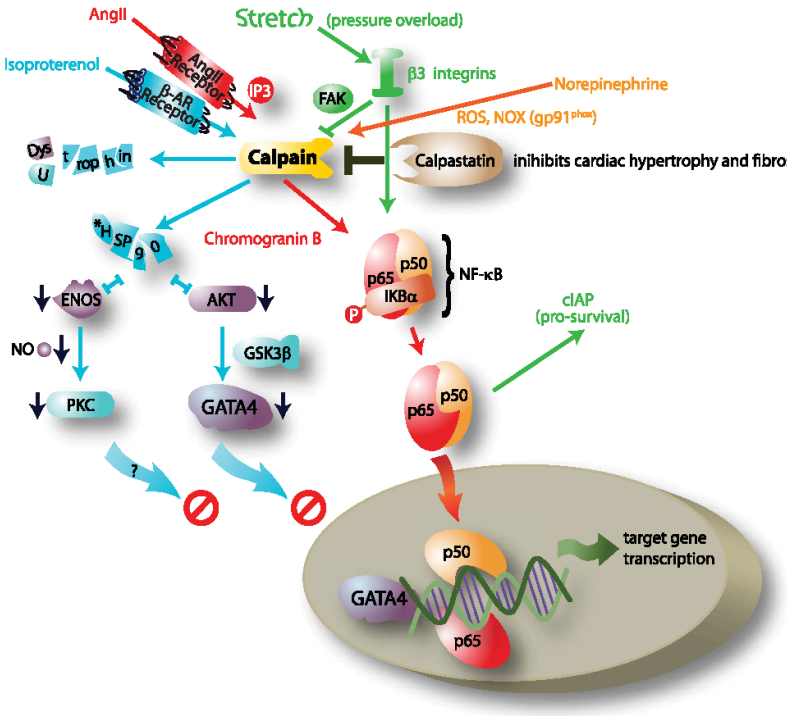

Figure 1. Compiled schema of calpain-related signaling during the development of cardiac hypertrophy

?=possible intermediates/connections not yet determined; FAK, focal adhesion kinase; HSP90, heat shock protein 90; GSK3β, glycogen synthase kinase 3 beta; GATA4, GATA binding protein 4; p65/p50, NF-κB heterodimer; NOX, NADPH oxidase; Ang II, angiotensin II. *indicates mechanisms identified in non-cardiomyocyte systems.

Table 1
Examples of calpain substrates relevant to cardiac (patho)physiology

Many calpain substrates have been described in non-muscle systems, with the exception of sarcomere proteins. Investigation of the role of calpain in muscle initially started with the realization of their role in meat "tenderization" (sarcomere breakdown), which is an active area of food research (see recent reviews[95-97]).

Substrate	References
Actin	98, 99
Amyloid precursor protein	100-102
Bax	103-105
Calcineurin	84
Caspases	31, 106
Ca^{2+} ATPase	107-109
Ca^{2+}/Calmodulin-protein kinase	110
c-Fos/c-Jun	111-113
Dystrophin, Utrophin, and Spectrin	70
Estrogen receptor	114, 115
Focal adhesion kinase	85, 86
G protein (α subunit)	116
IκBα	117, 118
Integrin β3	119-121
L-type Ca^{2+} calcium channel	48-50, 122, 123
p53	124, 125
Phospholipase C (PLC)	126, 127
Protein kinase A (PKA)	128
Protein kinase C (PKC)	129-131
Ryanodine receptors	132
Tau protein	133, 134

Table 2
Regulation of calpain and calpastatin activity and expression in cardiac disease

Cardiac Disease	Calpain Response	Calpastatin Response
Myocardial Infarction	N.D.	LV Free wall: Protein levels unaffected 1, 3, 7, 14 days after MI (Wistar Rats) [87]
Ischemia Ischemia/Reperfusion Injury	I: m-calpain translocates to the membrane m-calpain not activated with ischemia alone [42] I/R: m-calpain translocates to the membrane m-calpain activates in reperfusion [42]	Global I/R (20 min I/30 Min R): Calpastatin activity reduced ~40-60% [88] Protein levels reduced after reperfusion [88] Calpastatin protein levels decrease after I/R, but not after ischemia alone [42]
Congestive heart failure	MI induced heart failure: Calpain 1 and calpain 2 increased in viable LV muscle and RV muscle at 2 and 8 weeks. Calpain activities also increased [135] NYHA Class II: Increased calpain 1 protein levels. Calpain 2 levels not affected [54] NYHA Class III and IV: Increased Calpain 1 and Calpain 2 protein levels [54] Increased calpain 1 and calpain 2 protein expression [53]	MI induced heart failure: calpastatin protein levels and activity not changed at 2 and 8 weeks after MI [135]
Atrophy associated with mechanical unloading	Unloaded (transplanted) heart: Calpain 1 and 2 protein expression and activity levels increased [54]	N.D. N.D.

N.D. not determined.