Tear Me Down
Role of Calpain in the Development of Cardiac Ventricular Hypertrophy
Cam Patterson, Andrea L. Portbury, Jonathan C. Schisler, Monte S. Willis

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 the regulation of 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. (Circ Res. 2011;109:453-462.)

Key Words: cardiac hypertrophy | calpain | calpastatin | NF-kB inhibition | HSP90 heat-shock proteins | β3 integrin

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, epidemiological 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.5–10 Therefore, the reversal of cardiac hypertrophy therapeutically would likely involve decreasing protein synthesis, increasing the rate of protein degradation, or both. 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.

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 includes a series of enzymes that target specific substrate proteins for degradation by the 26S proteasome. The ubiquitin proteasome system–mediated regulation of cardiac mass has been shown to be mediated by multiple ubiquitin ligases, the components of the ubiquitin proteasome system that give it its specificity, as well as the proteasome. The ubiquitin ligases muscle ring finger-1 (MuRF1) and MAFbx (also known as atrogin-1) play a role in regulating cardiac mass,11–14 There is some evidence that suggests 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 which leaves 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. Inhibition of lysosome function in the heart results in an approximately 25% to 30% reduction in the overall rate of protein degradation.17 Although 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,

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nonlysosomal cysteine proteases that are expressed ubiquitously within all cells and whose function in muscle appears to involve both atrophic and hypertrophic pathways. 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, because a large body of work already exists describing the regulatory pathways involved in this form of proteolysis. The present 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 are tissue specific (see recent review by Bukowska et al.\(^2\)). Calpains are generally localized to the cytosol as inactive proenzymes 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 (Table 1). The conventional calpains, calpain 1 and 2 (also known as \(\mu\)- and m-calpain, respectively), are tightly regulated by an endogenous inhibitor called calpastatin.\(^2\) The 4 inhibitory domains of calpastatin bind reversibly to the active calpain domains to inhibit their activity. The activity of calpain is also inhibited by posttranslational modification by phosphate groups.\(^2\) For example, phosphorylation of Ser369 by protein kinase A prevents the formation of the active site necessary for calpain activity.\(^7\) Calpains have been implicated in degrading a diverse array of substrates involved in various areas of biology (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 line of muscle fibers, and have been associated with the in vitro degradation of sarcomeric proteins such as \(\alpha\)-tropomyosin.\(^7\) The majority of the studies that have examined calpain activity in the heart have focused on the role of this proteolytic system in response to pathological cardiac conditions such as postischemic cardiac injury,\(^35,79,80\) apoptosis (calpain 2),contraction, apoptosis (calpain 2), cell migration (calpain 1), cell differentiation (calpain 2), and cellular signal transduction in muscle (calpain 3).\(^2\)–\(^8\)

| Table 1. Examples of Calpain Substrates Relevant to Cardiac (Patho)physiology |
|-----------------|-------------------|
| Substrate       | References        |
| Actin           | 26, 27            |
| Amyloid precursor protein | 28–30         |
| Bax             | 31–33             |
| Calcineurin     | 34                |
| Caspases        | 35, 36            |
| Ca\(^{2+}\)/calmodulin-protein kinase | 40          |
| c-Fos/c-Jun     | 41–43             |
| Dystrophin, utrophin, and spectrin | 44            |
| Estrogen receptor | 45, 46         |
| Focal adhesion kinase | 47, 48        |
| G protein (\(\alpha\)-subunit) | 49               |
| Irbesartan     | 50, 51            |
| Integrin-\(\beta\)3 | 52–54        |
| L-type Ca\(^{2+}\) calcium channel | 55–59        |
| p53            | 60, 61            |
| Phospholipase C (PLC) | 62, 63       |
| Protein kinase A (PKA) | 64             |
| Protein kinase C (PKC) | 65–67         |
| Ryanodine receptors | 68              |
| Tau protein     | 69, 70            |

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).\(^7\)–\(^9\) Not calpain 2, is found to be active at physiological levels of calcium, which results in the proteolysis of specific substrates (eg, desmin and protein kinase Co), as well as increased protein ubiquitination and protein turnover by the 26S proteasome.\(^8\) 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, which suggests 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.\(^8\) Most interesting, 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 autophagosomes, and destruction of sarcomere integrity.\(^8\) 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), the accumulation of which 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).\(^8\)–\(^8\)
The involvement of calpain activity in the progression of cardiac pathologies is well known. 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). 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, which leads to the disruption of the excitation-contraction coupling mediated by Ca\(^{2+}\) channels. Likewise, calpain activation in reperfusion after ischemic insult has been proposed as a possible mechanism that mediates myocyte cell death, either by activation of apoptosis via Bid protein or through necrosis by increasing fragility due to degradation of sarcomeric proteins (recently reviewed by Insete et al). In isolated rabbit hearts exposed to global ischemic injury, calpain activity cleaves Bid, which results in the enhanced release of cytochrome C from mitochondria, which leads to apoptosis. There is also evidence for calpain involvement in congestive heart failure and in the atrophic remodeling that accompanies cardiac unloading. Ventricular tissue isolated from patients with congestive heart failure exhibits a marked increase in calpain expression. In milder cases of congestive heart failure (rated as class II on the New York Heart Association 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 (New York Heart Association classes 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. Likewise, in the unloaded rat heart, both calpain 1 and 2 expression and activity levels are increased, which provides further evidence of the involvement of calpain in the tissue remodeling associated with various cardiac pathologies.

### Calpain Regulation of Signaling in Cardiac Hypertrophy

The development of pathological 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 prohypertrophic gene expression programs (see recent reviews). Despite the complexity of these signaling pathways, only a relatively few transcription factors have been shown to drive this process, including NF-\(\kappa\)B (nuclear factor-\(\kappa\)B), GATA4 (GATA binding protein 4), NFAT (nuclear factor of activated T cells), SRF (serum response factor), and MEF2 (myocyte enhancer factor-2). The signaling pathways driven by these transcription factors facilitate hypertrophic growth of cardiomyocytes and activate so-called fetal genes. The concept of reexpressing genes normally expressed only during the fetal period of heart development is well established during the development of cardiac hypertrophy. Briefly, the activation of transcription factors such as SRF and GATA4 induces 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 (Ang II)—induced NF-\(\kappa\)B/NFAT pathway, the Akt signaling pathway, and the stretch-induced (\(\beta_3\)-integrin—mediated) signaling pathways. Interestingly, recent studies have implicated calpain in the regulation of cardiac hypertrophy via its specific interaction through each of these signaling pathways.

### Blocking Calpain Activity Disrupts Cardiac Hypertrophy by Inhibiting NF-\(\kappa\)B Activation

Ang II, a key component of the rennin-angiotensin-aldosterone system, induces cardiomyocyte hypertrophy by interacting with the Ang 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. 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-\(\kappa\)B signaling pathway. Infusion of Ang II leads to a robust increase in expression of the p65 subunit of NF-\(\kappa\)B in the nuclei of cardiomyocytes (which indicates enhanced activity), an effect that is considerably blunted in calpastatin transgenic mice. 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 an NFAT-independent, NF-\(\kappa\)B-dependent pathway.

The mechanism by which Ang II activates NF-\(\kappa\)B has been elucidated in recent studies published by Heidrich et al. 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\(_3\),R) pathway in cardiomyocytes. They found that the InsP\(_3\),R-dependent release of calcium, which turns on chromogranin B, leads to NF-\(\kappa\)B activation and expression of brain natriuretic peptide, a protein with increased expression in cardiac hypertrophy and heart failure. It has been postulated that calpains may mediate chromogranin activation in response to increased calcium in this system. NF-\(\kappa\)B activation may be related to chromogranin B activation as well. The evidence for this comes from studies that demonstrate an attenuated NF-\(\kappa\)B activity in cardiomyocytes with reduced chromogranin B expression. The proposed relationship of these signaling pathways is summarized in the Figure (highlighted in red). This Figure describes the relationship between the increased Ang II, a hormone increased in most patients developing cardiac hypertrophy, which then activates calpain activity, which results in enhanced downstream NF-\(\kappa\)B activity to induce the "prohypertrophic" genes in cardiomyocytes.

### \(\beta\)-Adrenergic Stimulation of Calpain Activity Blocks Endothelial Nitric Oxide Synthase and Akt Signaling

In addition to the role of calpain 1 in activating cardiac hypertrophy by activating NF-\(\kappa\)B, calpain activity can also
lead to inhibition of prohypertrophic signaling pathways. During the development of cardiac hypertrophy in humans, β-adrenergic stimulation occurs in parallel to stimulation by other G-protein–coupled receptors, such as the Ang II receptor. β-Adrenergic stimulation recently has been shown to activate calpains and block endothelial nitric oxide synthase (eNOS) activity and Akt signaling, both of which have been implicated as prohypertrophic signaling pathways. Experimentally, cardiomyocyte stimulation with the β-adrenergic agonist isoproterenol increases calpain activity while decreasing the activity of calpastatin. 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 (Figure, pathways in blue). In addition, a marked reduction in eNOS activity, a parallel decrease in heat-shock protein 90 (HSP90) levels, and an increase in caveolin 3 protein 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 to identify the link between increased calpain and decreased eNOS and Akt activity, it is possible that HSP90 may be a commonality between these 2 effects, as described below.

The interaction of HSP90 with eNOS and Akt enhances their activity. Experimentally, calpain 2 degrades HSP90 in culture. 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, likely through the HSP90-mediated enhancement of eNOS activity. Similar to the effects on eNOS, the HSP90/Akt complex formation is critical to Akt activity/phosphorylation. 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. Finally, isoproterenol administration in rats decreases HSP90 and eNOS activity in the heart at the same time Akt signaling is inhibited. Because both Akt-GATA4 and protein kinase C activation are crucial to the development of cardiac hypertrophy experimentally, the destabilization of HSP90 by calpain may be one mechanism that inhibits prohypertrophic signaling pathways in cardiomyocytes. The proposed mechanisms by which isoproterenol activates calpain to inhibit downstream eNOS and Akt signaling through its disruption of HSP90 are illustrated in the Figure (highlighted in blue).

**β3-Integrins Induce Calpain Activity to Enhance Cell Survival and Induce Cardiac Hypertrophy**

In the previous sections, we have discussed how Ang II stimulates calpain activity to enhance prohypertrophic signaling pathways via NF-κB and how β-adrenergic stimulation induces calpain activity to decrease eNOS/Akt stimulation, which possibly inhibits 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. Integrins are a class of receptors that extend through the plasma membrane and connect the intracellular sarcomere to
the extracellular matrix. 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. Recent studies have implicated integrin signaling in calpain activation and the development of cardiac hypertrophy. β3-Integrin–deficient mice that are subjected to transaortic constriction for 4 weeks to induce pressure-overload cardiac hypertrophy exhibit both an increase in cardiomyocyte cell death (by terminal dUTP nick end-labeling assay) and a decrease in ventricular mass compared with wild-type control mice. Pressure overload in β3-integrin–deficient mice also leads to an enrichment of calpain 1 in cardiac muscle, whereas pretreatment with calpeptin, a specific inhibitor of calpain, before pressure overload induction in β3-integrin–deficient mice attenuates the enhanced cell death as determined by terminal dUTP nick end-labeling staining. Although the role of calpain 1 in β3-integrin–mediated cardiac hypertrophy has not been determined definitively, it is possible that it serves a regulatory function to balance the processes of cell survival and cell death. 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 prosurvival factor cIAP (cellular inhibitor of apoptosis protein). The absence of these prosurvival signals (and therefore the unabated proapoptotic influence of calpain activation) in β3-integrin–deficient mice may account for the enhanced cardiomyocyte apoptosis seen during pressure-overload hypertrophy in these mice. Although much of this pathway remains to be elucidated, these studies demonstrate a link between the mechanically induced β3-integrins, calpain 1 activity, and maintenance of cell survival that possibly involves the prohypertrophic cIAP and NF-κB signaling as summarized in the Figure (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, which suggests that calpain activation may represent a general mechanism by which the cell responds to external stress, including stress hormones (norepinephrine, Ang II) 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 stimulation of adult rat ventricular cardiomyocytes via norepinephrine increases NADPH oxidase activity and reactive oxygen species generation, which leads to enhanced calpain 1 activation and apoptosis. Inhibition of the predominant NADPH oxidase in cardiomyocytes, gp91phox–NADPH oxidase, with apocynin or diphenyleneiodonium or inhibition of reactive oxygen species with the antioxidant N-acetylcysteine protects cardiomyocytes from apoptosis at the same time as it prevents the activation of calpain 1. Similarly, direct inhibition of calpain prevents cardiomyocyte apoptosis, presumably by blocking the norepinephrine-induced calpain activation that is mediated by NADPH oxidase. These studies indicate a central role of calpains that intersects with numerous diverse stress signaling pathways to activate cardiac hypertrophy (Figure, 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, which raises the obvious question of what calpain degradation of these proteins would 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 the induction of cardiac hypertrophy. 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. 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. Therefore, with the notable exception of calpain-mediated degradation of caspases, dystrophin, utrophin, spectrin, and the L-type Ca2+ channels 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 the present review focuses mainly on the role that calpains play in the regulation of cardiac ventricular hypertrophy, a brief discussion of 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 (Table 2). In the left ventricular free wall, calpastatin protein levels are not affected on days 1, 3, 7, and 14 after myocardial infarction in Wistar rats. Other studies have identified that ischemia reperfusion injury causes a downregulation of calpastatin activity. When hearts from Wistar rats were perfused ex vivo and challenged with a 20-minute period of global ischemia, followed by reperfusion for up to 30 minutes, calpastatin activity was reduced 40% to 60% when the rats were assayed for their ability to inhibit calpain 1 and calpain 2. Parallel decreases in protein levels of calpastatin were also identified after reperfusion (summarized in Table 2).
Table 2. Regulation of Calpain and Calpastatin Activity and Expression in Cardiac Disease

<table>
<thead>
<tr>
<th>Cardiac Disease</th>
<th>Calpain Response</th>
<th>Calpastatin Response</th>
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<tbody>
<tr>
<td>Myocardial infarction</td>
<td>ND</td>
<td>LV free wall: protein levels unaffected 1, 3, 7, 14 d after MI (Wistar rats)</td>
</tr>
<tr>
<td>Ischemia</td>
<td>Ischemia: m-calpain translocates to the membrane</td>
<td>Global I/R (20 min ischemia/30 min reperfusion): calpastatin activity reduced —40% to 60%</td>
</tr>
<tr>
<td>I/R injury</td>
<td>m-Calpain not activated with ischemia alone</td>
<td>Protein levels reduced after reperfusion</td>
</tr>
<tr>
<td></td>
<td>I/R: m-calpain translocates to the membrane</td>
<td>Calpastatin protein levels decrease after I/R, but not after ischemia alone</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>MI-induced heart failure: Calpain 1 and calpain 2 increased in viable LV muscle and RV muscle at 2 and 8 wk. Calpain activities also increased</td>
<td>MI-induced heart failure: calpastatin protein levels and activity not changed at 2 and 8 wk after MI</td>
</tr>
<tr>
<td></td>
<td>NYHA Class II: Increased calpain 1 protein levels. Calpain 2 levels not affected</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>NYHA Class III and IV: Increased Calpain 1 and Calpain 2 protein levels</td>
<td></td>
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<tr>
<td></td>
<td>Increased calpain 1 and calpain 2 protein expression</td>
<td></td>
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<tr>
<td>Atrophy associated with mechanical unloading</td>
<td>Unloaded (transplanted) heart: Calpain 1 and 2 protein expression and activity levels increased</td>
<td></td>
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</table>

ND indicates not determined; LV, left ventricular; MI, myocardial infarction; I/R, ischemia/reperfusion; RV, right ventricular; and NYHA, New York Heart Association.

A number of studies have been published that detail the effect of calpastatin overexpression, both systemically and specifically within the heart. Because 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, baseline cardiac functions (as determined by heart rate, heart work, and rate of contraction and relaxation) do not differ from those of wild-type mice. 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 breakdown products) or calpain 1 and calpain 2 expression. In unloaded hearts of mice overexpressing calpastatin, cardiomyocyte size also decreases, which suggests that other proteolytic systems may compensate for calpain activity. 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 β-adrenergic stimulation. 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 compared with wild-type mice. Similarly, isolated rat hearts in which calpastatin is overexpressed (via adenoviral transfection) exhibit a significant decrease in pathology associated with ischemia/reperfusion injury, as evidenced by greater left ventricular functional recovery and a decrease in degraded cardiac troponin I levels (a target of calpain degradation).

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. Control animals exhibited numerous physiological and pathological changes after 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, which strongly suggests the involvement of the calpain system in these cellular responses to the pressure overload and demonstrates 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 with animals that were not treated with the calpain inhibitor. 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 1 hour before treatment with isoproterenol, which suggests that calpain inhibition is effective in decreasing the effects of β-adrenergic–mediated cardiac hypertrophy. 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 remain to be determined.
Summary

The studies reviewed here largely demonstrate that inhibition of calpain activity during the induction of cardiac hypertrophy attenuates or prevents the development of hypertrophy, which suggests that calpains may be a novel target for treatment of 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 whether inhibition of calpain has any long-term side effects in the heart. The preclinical studies reported so far have not examined long-term outcomes of animals in which calpain inhibition prevented cardiac hypertrophy. Second, it must be determined whether inhibition of calpain activity in established pressure-overload–induced cardiac hypertrophy can reverse it enough to reduce the associated progression to heart failure and reduce the associated morbidity and mortality. Lastly, the effect of calpain inhibition on other organs (in both animals and humans) that would undoubtedly be affected by a systemic anticalpain approach must be studied. 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 (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 that make theoretically good targets for which synthetic inhibitors can be developed, from a medicinal chemistry point of view.135

The recent studies described in the present 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 the Figure). In addition, calpain activation mediates both prohypertrophic and antihypertrophic effects through NF-κB and eNOS/Akt signaling, respectively, although the contribution of each of these mechanisms in cardiac hypertrophy has not been elucidated completely. 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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Disclosures

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References


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