Calcineurin Inhibition in Hypertrophy
Back From the Dead!

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Reactive cardiac hypertrophy has long been recognized as a compensatory process that enhances ventricular function through normalization of wall stress in the face of increased hemodynamic load. For almost as long, it has also been appreciated that the natural history of a hypertrophied heart faced with unremitting hemodynamic overload is progression from a state of functional decompensation, or heart failure.1 Under these circumstances, the heart dilates and fails because hypertrophy fails, and in this manner, nature’s temporary cure for hemodynamic overload becomes part and parcel of the disease.

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Because a stimulus for hypertrophy is the initiator event in a reactive compensatory response leading to pathological decompensation, an obvious remedy for pathological myocardial hypertrophy is elimination of the stimulus. This is not always possible, however, owing to insufficient control of blood pressure in hypertensive patients, uncorrected valvular stenoses or insufficiencies, or hypertrophy of viable myocardium in postinfarction patients. Furthermore, genetic causes of hypertrophic cardiomyopathy are not directly addressable with current technologies. Therefore, identification of molecular and biochemical mediators of myocardial hypertrophy has been pursued to delineate hypertrophy signaling events that might be susceptible to targeted inhibition. Various candidate signaling pathways, mostly involving hormone receptors, associated G proteins, or their downstream kinase effectors, have been implicated with the use of in vitro and in vivo systems.2 Recently, protein phosphatases have also received attention in this regard.

The most thoroughly investigated and controversial protein phosphatase proposed to be a hypertrophy signaling factor is calcineurin (CN). CN is a ubiquitous phosphatase best known for its effects on T cell–mediated immunity. In these cells and others, sustained elevations in intracellular calcium activate CN, resulting in dephosphorylation of a class of transcription factors, nuclear factors of activated T cells (NFATs), which then translocate to cell nuclei and regulate expression of specific genes. CN is the target for 2 powerful immunosuppressive agents, cyclosporin A (CsA) and FK506, which have helped to revolutionize cardiac transplantation. Its role as a mediator of myocardial hypertrophy was unsuspected, however, until Molkentin et al3 identified CN in this context by using a yeast 2-hybrid screen for upstream mediators of cardiac GATA4 transcription factor activity. These investigators demonstrated that CN was capable of causing hypertrophy by transgenically expressing a constitutively active CN mutant (caCN) in the mouse heart. Striking cardiac hypertrophy developed by a few weeks of age, with progression to dilated cardiomyopathy and heart failure at 3 months.3 It was suggested that CN activation was not only sufficient to cause hypertrophy but also necessary for hypertrophy development because pharmacological inhibition with CsA and FK506 attenuated this response in angiotensin II– or phenylephrine-treated cultured cardiomyocytes and caCN mice.3 In a follow-up study, this same group of investigators showed that CsA and FK506 attenuated hypertrophy development in several (but not all) transgenic models of cardiac hypertrophy, as well as in aorta-banded rats.4

The above findings set off a flurry of studies in which CsA, FK506, or both were used to determine the role of CN signaling in various cardiac hypertrophy models. This level of investigative activity was likely prompted not only by the dramatic results reported by the Molkentin and Olson laboratories3–5 (and the obvious importance and potential clinical relevance of those findings, if true) but also by the relative ease with which such pharmacological inhibition experiments could be performed. The results of these studies have been reviewed in detail but can be summarized as being conflicting and, at times, contradictory. The confusion created by pharmacological CN inhibition studies has been attributed to technical variability, but it cannot be overlooked that the agents used to “specifically target” CN proved to be toxic at the levels used and that this toxicity must have confounded interpretation of the data. Thus, it has remained unclear whether CN is a key signaling intermediary in reactive myocardial hypertrophy or whether it is simply one of a growing number of signaling factors that are capable of causing hypertrophy when their activity in the myocardium is unrestrained.

Clarity on this issue required a new approach to CN inhibition, one that achieved specific functional inhibition without causing collateral systemic pathology. Recently published in vitro studies with the noncompetitive CN inhibitory peptides ΔCain and ΔAKAP79 demonstrated that CN inhibition by expressed proteins could prevent agonist-stimulated hypertrophy of cultured cardiomyocytes,6 suggesting the potential of a genetic approach. Now, in the span of a few months, 3 independent studies have been published that used...
the cleaner experimental design of transgenically expressing CN-inhibitory proteins in the mouse heart. Revisiting the role of CN in in vivo myocardial hypertrophy in this manner has substantially demystified its effects on myocardial growth.

In the current issue of Circulation, Zou and coworkers describe the effects of cardiac-specific transgenic expression of a dominant inhibitory CN mutant (dnCN) on pressure-overload hypertrophy in abdominal aorta–banded mice. In striking contrast to in vivo inhibition of CN with CsA, the dnCN transgene had no detrimental effects on unoperated mice. After aortic banding, nontransgenic mice exhibited increased cardiac CN activity (∼2-fold over baseline), confirming the association between hemodynamic stress, development of hypertrophy, and CN activation. This increase in CN activity was reduced by approximately two thirds in the aorta-banded dnCN mice, demonstrating in vivo inhibition of myocardial CN activity by the transgene. In a highly symmetrical experimental result, 3 weeks after banding there were also 60% to 70% decreases in several measures of cardiac hypertrophy, echocardiographic septal and posterior wall thicknesses, heart weight indexed to body weight, cardiomyocyte diameter, and the extent of left ventricular fibrosis. Interestingly, the characteristic elevations in atrial natriuretic peptide, brain natriuretic peptide, and c-fos gene expression seen with pressure overload were attenuated in the dnCN mice, but no increases in α-skeletal actin and c-jun mRNA were noted. Myocardial expression of dnCN was thus effective in inhibiting cardiac hypertrophy at the whole-organ, cellular, and molecular levels 3 weeks after short-term imposition of a pressure-overload stimulus.

A similar transgenic approach, with different CN-inhibitory peptides, was recently reported by De Windt et al in the Proceedings of the National Academy of Sciences USA. Two transgenic mouse strains were created, which expressed the ΔCain and ΔAKAP79 peptides previously shown to inhibit angiotensin- and phenylephrine-stimulated hypertrophy of cultured neonatal rat cardiomyocytes. Only mice with very low transgene copy numbers were viable, possibly due to inhibition of normal early postnatal developmental myocardial growth in mice with greater expression of the CN inhibitor. However, single- and double-transgene-copy ΔCain- and ΔAKAP79-transgenic mice were apparently normal at baseline. Hypertrophy was induced by long-term infusion of the β-adrenergic agonist isoproterenol or by abdominal aorta banding. In nontransgenic mice challenged in this manner, CN activity increased and myocardial hypertrophy developed, with an ∼25% increase in heart weight indexed to body weight. In ΔCain mice, the normal increase in CN activity after isoproterenol infusion was virtually abolished; in ΔAKAP79 mice, CN activity was halved after pressure overloading. Thus, these 2 CN-inhibitory peptides demonstrated the anticipated biochemical activities when expressed in myocardium. Transgenic cardiac expression of both CN inhibitors blunted the isoproterenol-mediated cardiac hypertrophy by ∼50% in both mouse strains. Differences in the extent of hypertrophy inhibition by the 2 peptides were observed 14 days after pressure overloading; however, whereas ΔCain diminished hypertrophy by ∼70%, ΔAKAP79 reduced it by only 25% to 38%. Thus, transgenic expression of peptide inhibitors of CN was highly effective in preventing CN activation after hormonal or mechanical stress and was partially effective in preventing the early hypertrophic response.

An especially strong feature of the report by De Windt et al is the use of in vivo adenoviral infection of rat myocardium to assess the effects of ΔCain, independent of developmental perturbations that are inevitable with transgenic expression that uses the α-myosin heavy-chain promoter. In effect, rats were treated with adenoviral ΔCain “gene therapy” that targeted CN and then underwent aortic banding. Seven days after pressure-overload modeling, the normal increase in CN activity was abolished by adenoviral ΔCain, and hypertrophy was diminished by 40%. These results show the potential for short-term, selective inhibition of CN to modify reactive myocardial hypertrophy.

In the same issue of the Proceedings of the National Academy of Sciences USA, Rothermel et al describe the effects of transgenic expression of a truncated form of the endogenous CN-inhibitory protein MCIP1 (myocyte-enriched CN-inhibitory protein 1). This natural inhibitor of CN is highly expressed in striated muscle and is transcriptionally upregulated as a consequence of CN activation. Thus, MCIP1 represents an endogenous negative regulatory mechanism for myocyte CN activity. When full-length MCIP1 cDNA was transgenically expressed in myocardium by using the α-myosin heavy-chain promoter, an unexpected RNA splicing event resulted in expression of a protein missing the amino terminal 80 amino acids but retaining full CN-inhibitory activity. Mice expressing lower levels of this protein exhibited a slight decrease in cardiac mass but were otherwise normal. When crossed with the caCN mice, the resulting compound-transgenic mice (expressing both activated CN and the CN inhibitor) were largely “rescued”; ie, hypertrophy was diminished by approximately three fourths compared with caCN littermates, and the characteristic early progression to dilated cardiomyopathy did not occur. Likewise, premature mortality in caCN mice was prevented by coexpression of MCIP1. Finally, MCIP1-transgenic mice had attenuated hypertrophic responses to long-term isoproterenol infusion and the physiological hypertrophy stimulus of unrestrained running. These studies demonstrate that an endogenous, naturally regulated inhibitor of myocardial CN signaling is effective in modulating cardiac hypertrophies resulting from unrestrained CN activity, catecholamine excess, and exercise.

These 3 nearly simultaneous reports of the effects of in vivo CN inhibition on cardiac hypertrophy indicate that CN signaling is necessary for myocardial growth in a variety of situations, both pathological and physiological. CN activity was inhibited by transgenic expression of 4 different proteins/peptides, with remarkably similar results. At levels of expression that resulted in viable mouse lines, there was little or no measurable effect of CN inhibition on baseline cardiac structure or function. Yet an important role for CN in normal postnatal cardiac developmental growth is strongly suggested by the dilated cardiomyopathy that occurred in the higher-expressing ΔCain mice and by the small decrease in cardiac
mass and inferential evidence of some embryonic lethality at higher expression levels in MCIP1 mice. Furthermore, inhibition of exercise-induced hypertrophy by MCIP1 supports a role for CN in a physiological adaptive response of fully developed hearts.

On the basis of these in vivo studies, it is difficult to dispute that CN has a critical role in the pathological hypertrophy response to catecholamine excess or pressure overload. Future investigations will determine whether there is a similarly important role in other forms of hypertrophy, particularly hypertrophic cardiomyopathy, wherein the hypertrophy stimulus is an intrinsic genetic defect that may or may not perturb intracellular calcium concentrations. It will also be necessary to perform experiments to determine whether hypertrophy signaling through other pathways may ultimately overwhelm inhibition of CN and result in a quantitatively normal but delayed hypertrophic response. Certainly, virtually complete CN inhibition incompletely prevented hypertrophy in one of the studies, indicating that CN-independent hypertrophy signaling pathways exist.

If one accepts a central role for CN activation in many forms of cardiac hypertrophy, what then are the therapeutic implications of these studies? It is obvious that CN inhibition can be achieved without the inescapable toxicity afforded by CsA and FK506. As experimental tools these agents were problematic, and as antihypertrophic agents it is not clear that a therapeutic window actually exists. These studies demonstrate that CN can be inhibited by large or small peptides, administered over either the short or long term. However, caution is warranted by the apparent inhibition of normal developmental and physiological hypertrophic responses with “superinhibition” of CN. An ideal “magic bullet” for hypertrophy should eliminate the pathology without ablating the beneficial aspects. After all, hypertrophy is an extremely effective means to diminish chamber wall stress and compensation for diminished intrinsic contractility or increased hemodynamic load; its adverse consequences primarily result from failure of the compensatory mechanism. Future studies will need to address the relative benefits of attenuating or inhibiting hypertrophy versus more subtly altering its fundamental characteristics, perhaps by modulation of signaling events that are not as central to myocardial growth as CN.

Acknowledgment
This study was supported in part by grants HL52318, HL58010, and HL/HD59888 from the National Institutes of Health, Bethesda, Md.

References

KEY WORDS: Editorial || cardiomypathy || hypertrophy || inhibitors || calcineurin
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Circulation. 2001;104:9-11
doi: 10.1161/01.CIR.104.1.9
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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