Control of Cardiac Growth and Function by Calcineurin Signaling*  

Published, JBC Papers in Press, July 24, 2003, DOI 10.1074/jbc.R300023200  

Rick B. Vega‡, Rhonda Bassel-Duby, and Eric N. Olson§  
From the Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, Texas 75390-9148

The central role of calcium in the control of cardiac function is well established. The release and reuptake of calcium by the sarcoplasmic reticulum (SR) controls not only the contraction of the cardiac myocyte but, ultimately, the heartbeat. However, the role of calcium as a key second messenger in signal transduction pathways that control growth of the heart has only recently been recognized. The calcium-dependent protein phosphatase calcineurin, also called protein phosphatase 2B, is a critical transducer of calcium signals that govern cardiac growth during development and disease. The actions of calcineurin are dependent on an array of effector proteins that influence its enzymatic activity, subcellular distribution, and stability. Additional proteins transmit calcineurin-dependent signals to the nucleus with consequent changes in gene transcription. Calcineurin signaling affects the functions of a wide range of cell types and although many of its effectors are ubiquitous, others are restricted to cardiac (and skeletal) muscle, providing muscle specificity to calcineurin signaling. The diversity of calcineurin effectors provides entry points into the signaling pathways that govern cardiac growth and function and provides opportunities for pharmacological and genetic modification of these processes. Here we discuss the functions of calcineurin and its effectors, the possibilities and pitfalls involved in its modulation as a therapeutic target in the heart, and questions for the future.

Calcium Signaling in Cardiac Contractility and Growth  

Changes in calcium signaling have short and long term effects on cardiac function (1). During cardiac contraction, or systole, cardiac uptake through L-type calcium channels in the sarcolemma induces intracellular calcium release from the SR by the ryanodine receptor (RyR) (Fig. 1). Calcium is then transported back into the SR during relaxation, or diastole, through the SR calcium-ATPase (SERCA2a). The activity of SERCA2a is controlled by phospholamban (PLB), a signal-responsive inhibitor of its activity. A growing body of evidence suggests that changes in calcium handling in response to pathological (e.g. pressure overload or ischemic damage) or physiological (e.g. exercise) stimuli influences not only the contractility but also cardiac growth (2). The adult heart grows by hypertrophy, which is mediated by an increase in myocyte size without an increase in myocyte number. Whereas hypertrophy in response to pathological stimuli is thought to be an initial salutary response to normalize ventricular wall stress and sustain cardiac output, chronic hypertrophy can progress to dilated cardiomyopathy with associated fibrosis, arrhythmias, and sudden death. Indeed, hypertrophy is a strong predictor for morbidity and mortality and is the single most important risk factor for heart failure in humans (3). Whether physiological hypertrophy and pathological hypertrophy are controlled by the same or different pathways is a central issue in the field with significant clinical implications because enhancing the former and suppressing the latter could have profound therapeutic consequences.

Calcium-dependent Activation of Calcineurin Signaling  

Calcineurin is a calcium/calmodulin-dependent serine/threonine protein phosphatase composed of a catalytic A subunit (CnA) and a regulatory B subunit (CnB) (reviewed in Ref. 4). It can be distinguished from the more abundant protein phosphatases 1 and 2A by its sensitivity to inhibition by the immunosuppressants cyclosporin A (CsA) and FK-506, and insensitivity to okadaic acid and calyculin A. Calcineurin is also unique in its specific responsiveness to sustained, low frequency calcium signals. The activation of calcineurin occurs through the binding of calcium/calmodulin, which displaces an autoinhibitory domain of the CnA subunit (Fig. 2).

The most well characterized substrate of calcineurin is the transcription factor nuclear factor of activated T-cells (NF-AT), so named because of its role in the activation of T-cells during the immune response (5). Calcineurin dephosphorylates multiple serine residues near the N termini of NF-AT proteins leading to their translocation from the cytoplasm to the nucleus where they engage a variety of transcription factors and activate calcineurin-responsive genes (6–8). Rephosphorylation of the same sites by glyco- gen synthase kinase-3β (GSK3β) and other kinases promotes nuclear export of NF-AT and terminates the calcineurin signal to the nucleus.

Calcineurin and Cardiac Hypertrophy  

Pathological cardiac hypertrophy is coupled to the activation of a “fetal” gene program in which proteins involved in contractility, calcium handling, and energy metabolism of the fetal heart are up-regulated with consequent changes in cardiac function. Members of the GATA and myocyte enhancer factor-2 (MEF2) families of transcription factors regulate, either directly or indirectly, fetal cardiac genes before birth and in response to stress signals in the adult heart (9, 10). The link between calcium and calcineurin as a key signal transduction pathway in the heart was first recognized by the discovery that NF-ATc4, one of four NF-AT proteins, associates with GATA4, a zinc finger transcription factor (11), thereby providing a potential connection between calcium-dependent signaling and fetal gene activation.

The initial description of calcineurin as a mediator of cardiac hypertrophy demonstrated that overexpression of an activated CnA subunit under control of the cardiac-specific α-myosin heavy chain (α-MHC) promoter in transgenic mice led to severe cardiac hypertrophy and eventually to heart failure and sudden death (11). This phenotype could be largely recapitulated by cardiac expression of a constitutively nuclear form of NF-ATc4. Subsequent studies demonstrated that inhibition of calcineurin activity with CsA and FK-506 suppressed hypertrophy in response to a variety of pathological stimuli, although conflicting results have been reported (summarized in Ref. 12). Targeted disruption of the gene encoding the CnBα isoform has also provided support for the role of calcineurin in pathological hypertrophy (13). CnBα null mice have smaller hearts than normal and show a markedly impaired hypertrophic response to infusion of angiotensin II or isoproterenol as well as to aortic constriction.

The measurable activity of calcineurin is elevated in response to several hypertrophic stimuli including β-adrenergic infusion, pressure overload, and exercise (14–16). Increased calcineurin activity...
Calcineurin and Calcium Handling in the Myocardium

During heart failure, signaling through the β-adrenergic pathway decreases. Normally, β-adrenergic signaling results in phosphorylation via protein kinase A of PLB, an inhibitor of SERCA2a. Phosphorylation of PLB relieves inhibition of SERCA2a and increases contractility (24). Calcineurin has been shown to dephosphorylate PLB in vitro (25). In addition, SERCA2a activity was decreased in human cardiac lysates in the presence of exogenous calcineurin. Paradoxically, however, the levels of phosphorylated PLB have been reported to be increased in the hearts of α-MHC-calcineurin transgenic mice (26); this was correlated with an increased calcium uptake by the SR and improved contractility in isolated cardiac myocytes. Calcineurin has also been shown to interact with the RyR in the SR in a calcium-dependent manner (27), although it was not detected by another group in an isolated RyR protein complex (28).

Csa treatment suppresses cardiac hypertrophy in response to a variety of hypertrophic stimuli. However, Csa treatment of a mouse model with a mutation in α-MHC resembling human familial hypertrophic cardiomyopathy resulted in decreased survival with a concomitant increase in heart size (29, 30). The mutant MHC protein has been proposed to act as an ion trap, decreasing SR calcium concentrations. If calcineurin promotes calcium uptake by the SR as discussed below, calcineurin inhibition by Csa may further decrease cardiac contractility of these mutant mice resulting in the observed decrease in survival.

Connections between Calcineurin and Other Hypertrophic Signaling Pathways

Connections between calcineurin and other signal transduction pathways governing cardiac myocyte growth may also contribute to the growth-promoting effects of calcineurin. Protein kinase C α, β1, and γ activation was found to be increased in the hearts of α-MHC-Cna transgenic mice (31). Furthermore, Csa prevented protein kinase C activation in rats following aortic constriction. Calcineurin also affects signaling through the mitogen-activated protein kinase pathway. Activation of c-Jun NH2-terminal kinase is enhanced whereas stress-responsive mitogen-activated protein kinase p38 activity is decreased by calcineurin (31, 32). Lower p38 activity correlated with increased protein levels of the dual specificity phosphatase MKP1, a protein thought to be responsible for p38 inactivation. Levels of ERK1/2 activation appear to be largely unaffected by calcineurin. In addition, p38 inhibition in vivo enhanced signaling through the calcineurin/NF-AT pathway suggesting cross-talk between the two pathways (33).

Calcineurin has also been shown to increase the activity of an unidentified kinase that phosphorylates class II histone deacetylases (HDACs) (34). Class II HDACs act as signal-responsive repressors of cardiac hypertrophy, at least in part by interacting with MEF2 and suppressing its ability to activate the fetal gene program. Phosphorylation of class II HDACs leads to their inactivation through nuclear export and promotion of the cardiac hypertrophic program. It is unclear, however, if calcineurin directly regulates the activity of this HDAC kinase or whether activation of the hypertrophic growth program leads indirectly to its enhanced activity, through autocrine feedback loops, for example.

Endogenous Modulators of Calcineurin

Recently, several endogenous protein inhibitors of calcineurin have been described. These include AKAP-79, cabin-1/cain, and the calcineurin B homology protein (35–37). In addition to their interest as biological modulators of calcineurin activity, these molecules have proven useful in studying the role of calcineurin in cardiac disease. Although these proteins are not expressed specifically in the heart, cardiac overexpression of the calcineurin inhibitory portions of the Cabin and AKAP-79 proteins blunted the hypertrophic response to isoproterenol infusion and aortic banding (16). Calcineurin modulators with defined functions in the cardiomyocyte are discussed below.
Calsarcins, appears to localize calcineurin to the Z-line of the sarcomere in skeletal and cardiac muscle (45, 46). Calsarcin-1 expression is limited to cardiac and skeletal muscle, whereas calsarcin-2 and -3 are skeletal muscle-specific. The interaction of calsarcin with α-actinin, an integral component of the Z-line, appears to be responsible for this localization. Both α-actinin and Ca2+ binding domains have been mapped on the calsarcin protein. The functional consequence of this subcellular localization of calcineurin is unknown; however, mutations in several Z-line proteins have been implicated in the development of dilated cardiomyopathy.

Glycogen Synthase Kinase-3
As described above, GSK3β antagonizes the actions of calcineurin by directly phosphorylating NF-AT and stimulating its nuclear export (47). Several hypertrophic stimuli have been shown to inhibit GSK3β activity, which would have the effect of augmenting calcineurin signaling. Transgenic mice that express a constitutively active form of GSK3β in the heart are resistant to hypertrophy in response to calcineurin activation, β-adrenergic infusion, and pressure overload (46). Whether all the anti-hypertrophic effects of GSK3β are mediated by NF-AT phosphorylation or whether other GSK3β substrates contribute to this effect remains to be determined.

Protein Kinase G
Recent studies have implicated the natriuretic peptide signaling pathway as a negative regulator of cardiac hypertrophy (48). Signaling by the natriuretic peptide receptors activates protein kinase G (PKG), which may represent a cardioprotective mechanism to suppress pathological signaling (49). Expression of activated PKG in cardiomyocytes also prevents hypertrophy in response to calcineurin activation. At least a portion of this effect can be ascribed to suppression of calcium entry through L-type calcium channels (50). PKG prevents nuclear translocation of NF-AT in response to hypertrophic agonists but not in response to constitutively activated calcineurin; this suggests that PKG acts both upstream and downstream of calcineurin in the hypertrophic signaling cascade.

Calcineurin and Cardiac Myocyte Apoptosis
Apoptosis of cardiac myocytes is triggered by a number of different pathological stimuli leading to heart failure (reviewed in Ref. 51), including ischemia/reperfusion injury, dilated cardiomyopathy, and hypertrophic cardiomyopathy. Calcineurin promotes apoptosis through dephosphorylation of the pro-apoptotic protein BAD (52). Inhibition of calcineurin by CsA or FK506 has been shown to limit the infarct size caused by ischemia/reperfusion injury to the heart (53). Furthermore, it was reported that calcineurin had pro-apoptotic properties in the heart (54). However, in a separate report calcineurin inhibition blocked the protective actions of endothelin-1 in response to hydrogen peroxide-induced apoptosis (55). Expression of the active form of calcineurin has also been shown to partially block the apoptotic effects of staurosporine and 2-deoxyglucose in cardiac myocytes (56). Most recently, a selective NFAT inhibitory peptide, VIVIT, was shown inhibit cardiac hypertrophy in neonatal cardiac myocytes in culture and to increase the degree of apoptosis observed in response to phenylephrine (57). The exact cardioprotective mechanism of calcineurin against myocyte apoptosis is unknown although it appears to require NFAT activation.

Calcineurin and Cardiac Arrhythmias
As previously discussed, overexpression of calcineurin in mice leads to extensive cardiac hypertrophy, heart failure, and sudden death (11). Electrocardiograms from these transgenic mice show recurrent episodes of sustained pleomorphic tachycardia prior to sudden death (58). Cardiac arrhythmias are an important mechanism contributing to the high mortality and sudden death of patients with cardiac ventricular hypertrophy. Many animal models of hypertrophy show a similar phenotype of prolonged action potential duration because of an increase in depolarization currents or a decrease in repolarizing currents (59). The molecular mechanisms that trigger these electrical changes are not known. Studies of transgenic mice overexpressing calcineurin show a decrease in the density of potassium channels (58). Inhibition of calcineurin activity in transgenic mice by administration of CsA not only reduced hypertrophy but also prevented a decrease in a rapidly
activating and inactivating, fast transient outward current. However, because of an observed decrease of the other outward currents by CsA treatment in wild-type animals, a reversal of the decrease in other outward currents was not evident. More work is needed to examine the effects of CsA treatment on action potential. A potential mode of action linking intracellular signaling to electrical remodeling and myocardial hypertrophy may involve calcineurin activity in phosphorylation modifications of ion channels. If this hypothesis is supported, therapeutic approaches to decreasing calcineurin activity may be applied to human electrophysiological pathologies.

Therapeutic Prospects and Pitfalls
The roles of calcineurin in cardiac disease including hypertrophy and failure make it an attractive therapeutic target. Pharmacological or genetic inhibition of calcineurin by overexpressing peptide inhibitors has a demonstrated benefit in preventing cardiac hypertrophy and failure in short term studies in animals. Whether inhibition of calcineurin activity is sufficient to suppress long term changes in the heart in response to stress signaling remains to be determined. The many physiological roles of calcineurin signaling, such as in the immune response, also pose significant challenges to its systemic inhibition as a means of blocking or reversing cardiac disease. Recent advancements in understanding the connections between the calcineurin and other hypertrophic signaling pathways, as well as new discoveries of effectors of calcineurin activity with cardiac specificity, should offer additional entry points into the cellular circuitry underlying cardiac hypertrophy and heart failure and will undoubtedly yield new approaches to therapeutic drug design for the treatment of heart disease.

Acknowledgment—We thank A. Tzenor for graphics assistance.

REFERENCES
1. Marks, A. R. (2003) J. Clin. Invest. 111, 597–609
2. Frey, N., McKinsey, T. A., and Olson, E. N. (2000) Nat. Med. 6, 1221–1227
3. Benjamín, E. J., and Levy, D. (1999) Am. J. Med. Sci. 317, 168–175
4. Rusnak, F., and Mertz, P. (2000) Physiol. Rev. 80, 1453–1521
5. Crabtree, G. R., and Olson, E. N. (1999) Cell 100, esp057–esp079
6. Beals, C. R., Clapham, N. A., Do, S. N., and Crabtree, G. R. (1997) Genes Dev. 11, 824–834
7. Jain, J., McCaffrey, P. G., Mizer, Z., Kerrpora, T. K., Lambert, J. N., and Kerppola, T. K. (1993) Nature 365, 352–355
8. Loh, C., Shaw, K. T., Carew, J., Viola, J. P., Luo, C., Perrino, B. A., and Rao, A. (1996) J. Biol. Chem. 271, 10884–10891
9. Passier, R., Zheng, Y., Huy, X., Yaya, F. J., Niel, R. L., McKinsey, T. A., Overbeek, P., Richardson, J. A., Grant, S. R., and Olson, E. N. (2000) J. Clin. Invest. 106, 1395–1406
10. Hasegawa, K., Lee, S. J., Jeon, S. M., Markham, B. E., and Kitsis, R. N. (1997) Circulation 96, 3943–3953
11. Molken, J. D., Lu, R., Antos, C. L., Markham, B., Richardson, J., Robbins, J., Grant, S. R., and Olson, E. N. (1998) Cell 93, 215–229
12. Molken, J. D. (2000) Circ. Res. 87, 731–738
13. Bueno, O. F., Wilkins, B. J., Tymitz, K. M., Glasscock, B. J., Kimball, T. F., Lorenzo, J. A., and Molken, J. D. (2000) Proc. Natl. Acad. Sci. U. S. A. 99, 4586–4591
14. Zou, Y., Tao, A., Zhu, W., Kudoh, S., Hori, Y., Shimoyama, M., Uozumi, H., Kohmoto, O., Takahashi, T., Shihabai, F., Nagai, R., Yazaki, Y., and Komuro, I. (2001) Circulation 104, 102–108
15. Zou, Y., Hori, Y., Uozumi, H., Takimoto, E., Teko, H., Zhu, W., Kudoh, S., Mizukami, M., Shimoyama, M., Shihabai, F., Nagai, R., Yazaki, Y., and Komuro, I. (2001) Circulation 104, 97–103
16. De Windt, L. J., Lim, H. W., Bueno, O. F., Liang, Q., Delling, U., Braz, J. C., Glasscock, B. J., Kimball, T. F., and Molken, J. D. (2001) Circulation 104, 1431–1437
17. Timmerman, L. A., Clapham, N. A., Ho, S. N., Northrup, J. P., and Crabtree, G. R. (1996) Nature 383, 837–840
18. Wilkins, B. J., De Windt, L. J., Bueno, O. F., Braz, J. C., Glasscock, B. J., Kimball, T. F., and Molken, J. D. (2002) Mol. Cell. Biol. 22, 7603–7613