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Does Contractile Ca^{2+} Control Calcineurin-NFAT Signaling and Pathological Hypertrophy in Cardiac Myocytes?

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Abstract

In noncontractile cells, a sustained increase in total cytoplasmic Ca^{2+} concentration is typically needed to activate the intracellular protein phosphatase calcineurin, leading to dephosphorylation of the transcription factor nuclear factor of activated T cells (NFAT), its nuclear translocation, and induction of gene expression. It remains a mystery exactly how Ca^{2+} -dependent signaling pathways, such as that mediated by calcineurin-NFAT, are regulated in contracting cardiac myocytes given the highly specialized manner in which Ca^{2+} concentration rhythmically cycles in excitation-contraction coupling. Here, we critically review evidence that supports the hypothesis that calcineurin-NFAT signaling is regulated by contractile Ca^{2+} transients in cardiac myocytes.

The primary function of the heart is to contract and pump blood in proportion to tissue needs. Each individual cardiac contraction (systole) depends on a transient increase in the cytosolic free Ca^{2+} concentration (the systolic Ca^{2+} transient, also commonly termed “contractile Ca^{2+} ”) to activate the contractile proteins that elicit pressure development and ejection of blood. Cardiac relaxation (diastole), in turn, depends on a decrease in the cytosolic free Ca^{2+} concentration. The frequency and amplitude of the systolic Ca^{2+} transient are regulated by neuroendocrine inputs and effectors to dynamically alter myocyte contractility and cardiac output (1). Alterations in contractility associated with such changes in the Ca^{2+} transient can be achieved by physiological stimuli, such as in response to exercise, or by pathologic stimuli, in response to hypertension or after myocardial infarction injury.

In general, pathologic disease states increase ventricular wall stress, necessitating an increase in contractile Ca^{2+} to maintain cardiac output. Persistent pathological stress on the heart also leads to cardiac hypertrophy, circumstantially linking increases in contractile Ca^{2+} with pathological cardiomyocyte growth. Various physiologic stimuli, such as adolescent development, pregnancy, and aerobic exercise training, also enhance cardiac output and contractile Ca^{2+} in association with hypertrophic growth. Therefore, both physiological (exercise) and pathological (hypertension) stimulation elicit increases in contractile Ca^{2+} , and both conditions lead to cardiac hypertrophy. However, physiological and pathological hypertrophy are likely transduced by fundamentally different signaling pathways, despite the commonality of enhanced contractile Ca^{2+} (2).

Regulation of contractile Ca^{2+} in cardiac myocytes involves a highly specialized system of ion channels, pumps, exchangers, and microdomains (1). Contraction starts when depolarization

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of the sarcolemma (plasma membrane) stimulates the opening of voltage-gated L-type Ca^{2+} channels within the transverse (T) tubules (invaginations of the sarcolemma) and Ca^{2+} enters a sub-membrane microdomain abutting the junctional sarcoplasmic reticulum (SR) (Fig. 1). Ca^{2+} concentration increases within this microdomain are greater than those in the bulk cytoplasm and are required to stimulate the opening of Ca^{2+} release channels (ryanodine receptors) in the SR membrane and the subsequent release of Ca^{2+} from the SR, which, in turn, increases bulk intracellular Ca^{2+} concentration more than 10 times. It is this increase in bulk Ca^{2+} that stimulates contraction. Relaxation occurs when Ca^{2+} is taken back up into the SR through the action of the SR Ca^{2+} adenosine triphosphatase (ATPase) (SERCA) and is extruded from the cell by the sarcolemmal Na^+ and Ca^{2+} exchanger (Fig. 1).

Changes in contractile Ca^{2+} are thought to coordinate the activity of various Ca^{2+} -dependent signaling molecules, including protein kinase C (PKC), Ca^{2+} -calmodulin-dependent protein kinase (CaMK), and the Ca^{2+} -activated protein phosphatase calcineurin (also known as PP2B). Two scenarios have emerged to explain how changes in cardiomyocyte Ca^{2+} concentration could regulate specific signaling pathways in the backdrop of cyclic Ca^{2+} transients. The first scenario postulates the existence of specialized cellular microdomains in which Ca^{2+} concentration is locally regulated and sensed by macro-molecular signaling complexes housed in such regions. This scenario posits that microdomain Ca^{2+} concentration is largely independent of the bulk contractile Ca^{2+} . Thus, the systolic Ca^{2+} transient has no effect on hypertrophic signaling pathways in this first scenario. The second proposes that the same pool of contractile Ca^{2+} that controls cardiac contractility underlies activation of several specific signaling pathways, such that greater inotropy leads to greater reactive signaling. However, this latter scenario may be untenable, because the Ca^{2+} activation requirements for calcineurin, CaMK, and PKC are each unique and not consistent with the changes in Ca^{2+} that occur during the contractile cycle. (3–5).

Alterations in the amplitude of the systolic Ca^{2+} transient associated with physiological or pathological stimulation are often brought about by catecholamines (released from sympathetic nerves) acting through the β -adrenergic receptor. Catecholamines increase the magnitude of the Ca^{2+} transient through a cyclic adenosine 3',5'-monophosphate (cAMP)–protein kinase A (PKA) signal transduction cascade that results in phosphorylation of Ca^{2+} -handling channels and pumps (Fig. 1).

It is not yet known whether stress-induced increases in contractile Ca^{2+} regulate the activity of Ca^{2+} -sensitive signal transduction pathways, such as the calcineurin signaling cascade, in mediating pathological hypertrophy. Calcineurin activation induces pathologic cardiac hypertrophy and deleterious remodeling of the heart through activation of the transcription factor nuclear factor of activated T cells (NFAT) (6,7). This calcineurin-NFAT signaling module does not appear to be activated during exercise or pregnancy-induced physiologic hypertrophy (8), even though contractile Ca^{2+} is increased. Hence, proposing that contractile Ca^{2+} controls calcineurin-NFAT signaling in vivo would imply that cardiomyocytes can differentiate between physiologic (exercise) and pathologic increases in contractile Ca^{2+} .

Despite these issues, a number of investigators have suggested that contractile Ca^{2+} directly controls calcineurin-NFAT signaling in cardiac myocytes. A recent paper by Pozzan and colleagues provides additional evidence to support this hypothesis (9). The authors showed that, in neonatal rat ventricular myocytes, angiotensin II, which is known to activate cardiac hypertrophy in combination with calcineurin signaling (10), produced an increase in the frequency of contractile Ca^{2+} transients, leading to an increase in the time-averaged bulk cytosolic (contractile) Ca^{2+} (9). Similarly, addition of KCl to the culture media to depolarize the plasma membrane increased Ca^{2+} transient frequency and produced a hypertrophic response as assessed by measurement of cell area (9). The hypertrophy associated with KCl

was blocked by an inhibitor of the L-type Ca^{2+} channel, which reduces Ca^{2+} influx, but not by a generalized inhibitor of contraction that functions at the level of the myofilament (9). Similar results were observed with other agents that also increased contractile Ca^{2+} , such as norepinephrine and aldosterone. These same agonists and their associated increases in Ca^{2+} transient frequency and total Ca^{2+} also induced NFAT nuclear translocation in neonatal rat cardiomyocytes (9). Even increasing Ca^{2+} transient frequency by field stimulation was sufficient to activate NFAT nuclear translocation in these cultured primary cells (9).

These results are consistent with studies from other laboratories. For example, increasing the beating frequency of cultured neonatal cardiomyocytes or atrial preparations stimulates NFAT translocation to the nucleus (11,12). These results are similar to those obtained with cultured skeletal muscle myofibers, in which increased pacing frequencies progressively enhanced NFAT translocation from the sarcomeres to the nucleus, suggesting again that contractile Ca^{2+} is capable of activating calcineurin signaling (13,14). Similarly, pacing and KCl depolarization activated calcineurin enzymatic activity and caused NFAT translocation to the nucleus in cultured primary and C2C12 myotubes (15). Collectively, these results in cultured cardiomyocytes and skeletal myofibers suggest a paradigm in which increases in contractile Ca^{2+} can mediate calcineurin activation and NFAT nuclear translocation, which, in cardiomyocytes, presumably induces the pathologic hypertrophic response.

The studies described above in which pacing, agonist stimulation, or KCl-mediated depolarization increased contractile Ca^{2+} and caused calcineurin-NFAT activation were all performed in ex vivo experimental systems, usually in cultured cells and often in neonatal myocytes. One concern is that microdomains in which Ca^{2+} -mediated signaling might take place could be very different in adult myocytes in vivo compared with cultured neonatal myocytes, as could the molecules that partner with Ca^{2+} to activate physiological versus pathological signaling. Therefore, whether contractile Ca^{2+} directly activates calcineurin-NFAT signaling in the adult heart remains unclear. Indeed, experiments in mice indicate that deletion of the gene encoding phospholamban (PLN), which controls the activity of SERCA2, increases SR Ca^{2+} loading, contractile Ca^{2+} transients, and contractility yet does not stimulate pathologic (or physiological) cardiac remodeling or hypertrophy (16). Thus, increasing total contractile Ca^{2+} in a normal mouse heart does not seem to activate calcineurin-NFAT signaling. Overexpressing components of the L-type Ca^{2+} channel in transgenic mice, which increases contractile Ca^{2+} , does induce pathological hypertrophy and dilatory remodeling (17,18). However, this phenotype is associated with Ca^{2+} overload and necrotic myocyte death, likely leading to a secondary hypertrophy response. Therefore, a direct link between increased contractile Ca^{2+} and pathological hypertrophy still cannot be made in vivo. In human heart failure, there are complex changes in the regulation of contractile Ca^{2+} , with decreased rates of Ca^{2+} reuptake by the SR, prolongation of the Ca^{2+} transient, increased diastolic Ca^{2+} (19), and progressive negative remodeling of the ventricles. Calcineurin-NFAT signaling is activated in this scenario (20).

Heart failure is a very complex syndrome, which also involves neuroendocrine stimulation that could partner with Ca^{2+} signaling pathways within microdomains to induce pathological hypertrophic signaling. For example, the few L-type Ca^{2+} channels that reside within caveolin-rich sarcolemma microdomains in association with signaling effectors (Fig. 1) could be involved in activating calcineurin-NFAT without directly contributing to contractile Ca^{2+} (21). A Ca^{2+} channel-dependent microdomain signaling system in the adult heart could be independent of the bulk cytoplasmic Ca^{2+} but would still be responsive to many of the same factors that regulate Ca^{2+} flux into the T tubular-SR microdomain that is responsible for excitation-contraction coupling and contractile Ca^{2+} . Thus, we remain uncertain whether contractile Ca^{2+} serves as a direct mediator of pathologic hypertrophy and calcineurin-NFAT signaling within the intact heart.

Translating mechanistic relations between contractile Ca^{2+} and the hypertrophic response from experiments performed in neonatal cardiomyocyte cultures to the adult heart must be done cautiously because Ca^{2+} handling in these two contexts is substantially different. For example, cultured neonatal myocytes lack T tubules and an organized junctional microdomain for SR coupling (22). Moreover, calcineurin and NFAT are found in a diffuse pattern throughout the cytosol of cultured neonatal myocytes, whereas in adult myocytes both proteins are anchored at the Z lines overlying the T tubules, a completely different Ca^{2+} -sensing environment (23). Calcineurin-NFAT and the hypertrophic response have been associated with pacing of intact adult atria and during atrial fibrillation (24,25). Both of these conditions may cause such large changes in contractile Ca^{2+} , particularly in diastolic Ca^{2+} , that even protected Ca^{2+} -signaling microdomains that regulate hypertrophic signaling could become “contaminated” with bulk cytoplasmic Ca^{2+} . These issues remain largely unresolved.

Another issue surrounding various studies in cultured neonatal cardiomyocytes is that they fail to directly implicate exactly how contractile Ca^{2+} promotes calcineurin-NFAT activation and the pathologic hypertrophic growth response. For example, the data of Pozzan and colleagues, as well as that of Tavi *et al.* in atrial preparations, could be interpreted in two different ways (9,11). Both groups showed that increasing the frequency at which Ca^{2+} transients occur activates calcineurin-NFAT signaling and the hypertrophic response. However, increasing the rate of Ca^{2+} transients also increases total averaged Ca^{2+} within the cytosol. Thus, the conclusion that hypertrophy and calcineurin-NFAT are regulated by rates of Ca^{2+} transients is uncertain because changes in diastolic and total averaged Ca^{2+} are rate-dependent. Indeed, simply prolonging the action potential in cultured myocytes with dominant negative effectors of the transient outward K^+ current, which secondarily increases total net Ca^{2+} within the cytosol, induces hypertrophy through activation of calcineurin-NFAT signaling (26). Conversely, shortening action potential duration by overexpression of select K^+ channels reduces total cytosolic Ca^{2+} concentrations and blocks hypertrophy of myocytes in culture, which are associated with reduced calcineurin-NFAT signaling (27,28). Thus, the results in cultured neonatal myocytes fail to definitively implicate what portions of contractile Ca^{2+} (rate of rise, peak systolic Ca^{2+} , time-averaged Ca^{2+} , or diastolic Ca^{2+}) might elicit hypertrophy and calcineurin-NFAT signaling. Despite these detailed issues, the greater issue pertaining to the overall relevance of results obtained in cultured neonatal myocytes is more problematic given near-complete absence of microdomains associated with T tubules, the sarcolemma, and SR compartments.

We favor a scenario in which Ca^{2+} regulation of calcineurin-NFAT signaling and resultant pathological hypertrophy occurs in spatially localized and regulated subcellular microdomains. The fact that contractile Ca^{2+} is increased in both physiological and pathological cardiovascular stress and that calcineurin-NFAT signaling is only activated in pathological conditions argues for microdomain regulation. Experiments showing that CaMKII is regulated by a perinuclear Ca^{2+} pool associated with localized inositol 1,4,5-trisphosphate receptor (IP_3R) activity, which upon activation regulates translocation of histone deacetylase 5 (HDAC5) out of the nucleus to presumably permit hypertrophic gene expression (29), indicate that such a paradigm is possible in the heart. The sarcolemma and T tubules of adult cardiac myocytes are rich in caveolin and could be sites of microdomain Ca^{2+} signaling that lead to localized activation of calcineurin and NFAT (Fig. 1). The fact that the loss of *caveolin-1* and *caveolin-3* by gene targeting in the mouse induces hypertrophic cardiomyopathy and the unrestrained activation of signaling pathways support this hypothesis (30). It is likely that neuroendocrine or stretch-dependent signaling receptors, or both, directly communicate with these presumed Ca^{2+} microdomains to induce the hypertrophic response and activation of Ca^{2+} -dependent signaling effectors. Elucidation of additional Ca^{2+} signaling microdomains in adult cardiac myocytes will be important in resolving how the myocyte parses signaling versus contractile Ca^{2+} .

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Fig. 1.

Model of the T tubule and SR with calcium-handling proteins and its relation with β -adrenergic receptor signaling. A small amount of priming Ca^{2+} enters through the L-type Ca^{2+} channel (LTCC) to stimulate the ryanodine receptor (RyR) within the junctional complex microdomain (pink area). This induces RyR2 to open, allowing release of larger quantities of Ca^{2+} from the sarcoplasmic reticulum into the bulk cytoplasm and stimulating the myofilament proteins to induce contraction. Relaxation is produced by reestablishing a low concentration of cytosolic Ca^{2+} through closure of LTCC and RyRs, Ca^{2+} transport into the SR through SERCA, and Ca^{2+} efflux through the $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger (NCX). β -adrenergic receptors (β -ARs) respond to catecholamines, resulting in activation of adenylyl cyclase (AC), generation of cAMP, and the activation of PKA. PKA then phosphorylates LTCC, RyR2, and PLN to enhance contractile Ca^{2+} cycling and contractility. Cardiac myocytes also contain lipid rafts rich in caveolin in the sarcolemma and T tubules that are proposed to generate local microdomains of Ca^{2+} to regulate hypertrophic signaling and activation of Ca^{2+} signaling effectors such as calcineurin.