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Original article

Ca^{2+} influx through L-type Ca^{2+} channels and transient receptor potential channels activates pathological hypertrophy signaling

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ABSTRACT

Common cardiovascular diseases such as hypertension and myocardial infarction require that myocytes develop greater than normal force to maintain cardiac pump function. This requires increases in $[Ca^{2+}]$. These diseases induce cardiac hypertrophy and increases in $[Ca^{2+}]$ are known to be an essential proximal signal for activation of hypertrophic genes. However, the source of "hypertrophic" $[Ca^{2+}]$ is not known and is the topic of this study. The role of Ca^{2+} influx through L-type Ca^{2+} channels (LTCC), T-type Ca^{2+} channels (TTCC) and transient receptor potential (TRP) channels on the activation of calcineurin (Cn)–nuclear factor of activated T cells (NFAT) signaling and myocyte hypertrophy was studied. Neonatal rat ventricular myocytes (NRVMs) and adult feline ventricular myocytes (AFVMs) were infected with an adenovirus containing NFAT–GFP, to determine factors that could induce NFAT nuclear translocation. Four millimolar Ca^{2+} or pacing induced NFAT nuclear translocation. This effect was blocked by Cn inhibitors. In NRVMs Nifedipine (Nif, LTCC antagonist) blocked high Ca^{2+}-induced NFAT nuclear translocation while SKF-96365 (TRP channel antagonist) and Nickel (Ni, TTCC antagonist) were less effective. The relative potency of these antagonists against Ca^{2+} induced NFAT nuclear translocation (Nif > SKF-96365 > Ni) was similar to their effects on Ca^{2+} transients and the LTCC current. Infection of NRVM with viruses containing TRP channels also activated NFAT–GFP nuclear translocation and caused myocyte hypertrophy. TRP effects were reduced by SKF-96365, but were more effectively antagonized by Nif. These experiments suggest that Ca^{2+} influx through LTCCs is the primary source of Ca^{2+} to activate Cn–NFAT signaling in NRVMs and AFVMs. While TRP channels cause hypertrophy, they appear to do so through a mechanism involving Ca^{2+} entry via LTCCs.

1. Introduction

Pathological cardiac stress, such as hypertension and myocardial infarction (MI), causes alterations in cardiac myocyte Ca^{2+} handling [1,2]. The increase in cytosolic Ca^{2} activates the calcineurin (Cn)–nuclear factor of activated T cells (NFAT) signaling pathway. Cn-mediated dephosphorylation of NFAT leads to its nuclear translocation where it initiates the expression of genes involved in pathological cardiac hypertrophy. Blocking this pathway has beneficial effects on cardiac structure and function [3–5]. However, the upstream sources of Ca^{2+} that initiate pathological hypertrophy through Cn–NFAT signaling are still not clearly established. Recent studies suggest that Cn–NFAT activation may be independent of the global cardiac Ca^{2+} transient and may take place in signaling microdomains also housing T-type Ca^{2+} channels [6,7] or transient receptor potential (TRP) channels [8–11]. Other studies have suggested that Cn–NFAT signaling is sensitive to changes in the rate and amplitude of the systolic Ca^{2+} transient [12,13]. A recent study from our group suggests that L-type Ca^{2+} channels housed in caveolae, away from excitation–contraction coupling proteins are a local microdomain for Ca^{2+}-activation of Cn–NFAT signaling [14].

The role of Cn–NFAT signaling in the induction of pathological hypertrophy was first proposed by Molkentin et al. [15]. This study showed that when a constitutively activated Cn was expressed in cardiac myocytes of transgenic mice, pathological cardiac hypertrophy developed and resulted in heart failure. Many studies have since shown that this pathological Cn–NFAT signaling pathway is activated by increases in Ca^{2+}. When the intracellular Ca^{2+} concentration is elevated, Ca^{2+} ions bind to calmodulin (CaM) and the Ca^{2+}/CaM complex activates the protein phosphatase Cn. Activated Cn subsequently dephosphorylates its downstream substrate, NFAT. Dephosphorylated NFAT translocates from the cytosol into the nucleus and activates transcription factors (e.g. GATA4), which initiate hypertrophic gene

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expression [16]. The source of the Ca^{2+} that activates pathological Cn–NFAT signaling is still not well known and is the topic of this study.

One possible source of Ca^{2+} that activates the Ca^{2+}/CaM–Cn–NFAT pathway in the heart is the high-conductance, voltage-regulated L-type Ca^{2+} channels (LTCC). These channels provide the Ca^{2+} influx that is essential for inducing and regulating myocyte contraction. This Ca^{2+} influx is essential for triggering sarcoplasmic reticulum (SR) Ca^{2+} release (CICR) [17]. There is some evidence that Ca^{2+} entry through the LTCC can induce pathological hypertrophy [18,19]. In myocytes isolated from animal models of hypertrophy [20–24] or from failing human hearts [25], the L-type Ca^{2+} current density is unchanged or slightly elevated [26]. However, these measurements are made in the absence of the sympathetic agonists that increase Ca^{2+} current and are increased in cardiovascular diseases [27–29]. Therefore, in-vivo, LTCC current is increased in disease and is a possible source of Ca^{2+} to activate pathological signaling [30–34]. Further support for a role for LTCCs comes from studies that have shown beneficial effects of LTCC antagonists on cardiac remodeling in distinct hypertrophy and heart failure models [5]. However there are numerous studies suggesting that other Ca^{2+} influx pathways are the primary source of "hypertrophic" Ca^{2+}. Experiments in the present study support the idea that LTCCs are the primary source of Ca^{2+} to activate Cn–NFAT signaling in NRVMs and AFVMs.

Ca^{2+} can also enter cardiac myocytes through T-type Ca^{2+} channels (TTCC), which are normally expressed throughout cardiac development until the end of the neonatal period [35–37]. TTCC expression decreases after birth with little expression in normal adult ventricular myocytes. However, TTCCs are re-expressed when the heart is subjected to pathological stresses, suggesting a role in cardiac hypertrophy and failure [38–42]. Ca^{2+} influx through TTCC has almost no effect on EC coupling [43]. Cav3.1 (α1G) and Cav3.2 (α1H) are the major TTCC isoforms in the heart [44]. A few studies support the idea that Ca^{2+} influx through TTCCs can activate Cn–NFAT signaling and cause hypertrophy in neonatal cardiac myocytes, when TTCC expression is robust [7]. There are also studies supporting the idea that Ca^{2+} influx through TTCC causes pathological hypertrophy in the adult heart. One study [45] in a mouse model of dilated cardiomyopathy, reported that the R(−)-isoform of efonidipine, a highly selective TTCC antagonist improved survival. Another study [6] showed that pressure overload-induced hypertrophy was suppressed in Cav3.2 knockout (Cav3.2 −/−) mice. Angiotensin II-induced cardiac hypertrophy was also reduced in these mice and the activation of NFAT was blunted following pressure overload. However, there are other studies that do not support a roll of TTCC as a source of Ca^{2+} for cardiac hypertrophy [43,46]. Mice overexpressing Cav3.1 and with large TTCC currents did not have cardiac hypertrophy [43]. In fact, when these animals were subjected to pathological stress their hypertrophy was smaller than in wild type animals, suggesting that Ca^{2+} influx through TTCCs is antihypertrophic [46]. The role of Ca^{2+} influx through TTCCs in pathological hypertrophy remains unresolved.

Ca^{2+} can also enter cardiac myocytes through Transient Receptor Potential (TRP) channels. TRP channels are nonselective cation channel subunits responsible for receptor activated Ca^{2+} entry and possibly having a role in store operated Ca^{2+} entry (SOCE) [47]. TRP channels have been grouped into 7 subfamilies [48,49]. Several studies have reported that the canonical TRP channels (TRPC) are involved in cardiac hypertrophy and heart failure [49]. TRPC have 7 family members and are divided into 2 subgroups based on their primary amino acid sequences and functions. TRPC1, C3–7 are expressed in the heart [50,51] and TRPC3 expression is up-regulated in multiple rodent models of pathological cardiac hypertrophy [52]. There is some evidence that TRPC promotes cardiomyocyte hypertrophy through activation of Cn–NFAT signaling [9] This effect was blocked by the store operated Ca^{2+} entry (SOCE) antagonist SKF-96365. Small interference RNA-mediated knockdown of TRPC3 and TRPC6 has also been shown to attenuate Ang II-induced NFAT activation and myocyte hypertrophy [10]. Cardiac-specific overexpression of TRPC6 in transgenic mice sensitizes the heart to pathologic hypertrophic signaling, leading to cardiac dysfunction, hypertrophy and increased β-myosin heavy chain expression, which is regulated by Cn–NFAT signaling [11]. Collectively these studies suggest that Ca^{2+} influx through TRP channels can activate pathological hypertrophy signaling.

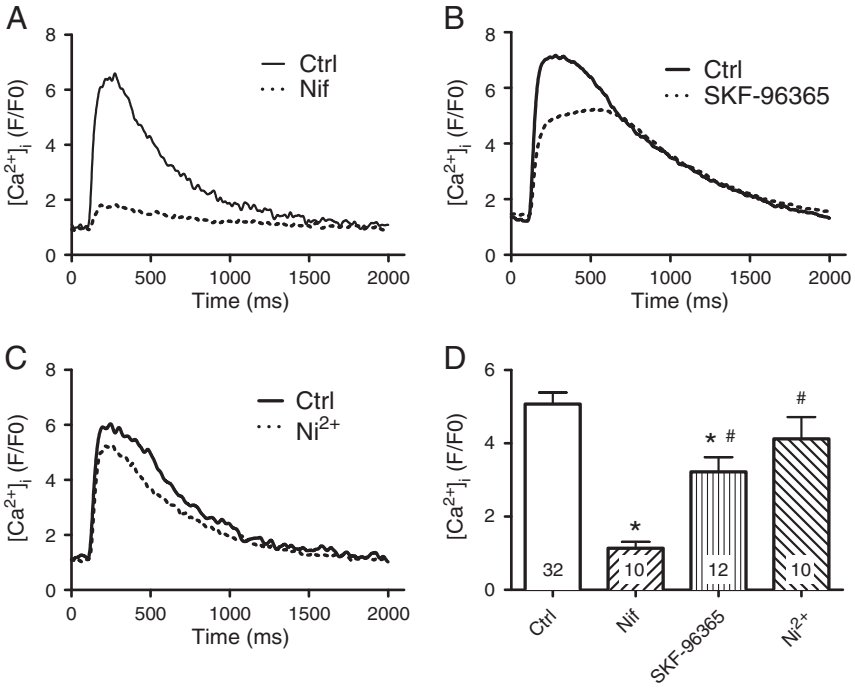

Fig. 1. Nifedipine, SKF-96365, and Ni^{2+} have distinct effect on Ca^{2+} transients. Ca^{2+} transients were measured in NRVMs perfused with normal Tyrode's solution (Ctrl) before and after exposure to solutions containing either 10 μM Nifedipine (Nif, A), 5 μM SKF-96365 (B), or 50 μM Ni^{2+} (C). Peak Ca^{2+} was reduced 79%, 33%, and 20% by these treatments, respectively (D). * indicates a significant treatment effect versus control. # indicates significant difference versus Nif. $P<0.05$. Numbers in bars represent the number of cells studied.