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Calcineurin Finds a New Partner in the L-Type Ca^{2+} Channel

Geoffrey S. Pitt

Calcineurin is a Ca^{2+}/calmodulin-sensitive phosphatase that sits near the top of signaling pathways leading to pathological cardiac hypertrophy.[1] Pathological stressors activate calcineurin, for which nuclear factor of activated T cells (NFAT) is a principal substrate. Dephosphorylation unmasks a nuclear localization signal on NFAT, which then translocates to the nucleus and, there, serves as part of a transcription factor complex able to initiate gene expression cascades that induce cardiac hypertrophy and subsequent heart failure. Determining how calcineurin is specifically activated in response to hypertrophic stressors by the same Ca^{2+} signal used for excitation–contraction coupling has been a major challenge. Triggered by Ca^{2+} influx through $Ca_V1.2$ voltage-gated Ca^{2+} channels at the sarcolemmal membrane, intracellular calcium briefly increases 10-fold with each heart beat to generate myocyte contraction. Almost as quickly, intracellular calcium falls to its baseline level, allowing myocyte relaxation and preparation for the next beat.

There are 2 leading proposals for how Ca^{2+} could activate the calcineurin/NFAT pathway or function as a versatile regulator of multiple other signaling cascades in the face of the large Ca^{2+} oscillations driving each cycle of contraction and relaxation. One is that modulation of the amplitude and/or frequency of the oscillatory contractile Ca^{2+} signal triggers particular Ca^{2+} sensors that then activate particular downstream signaling pathways, such as calcineurin activation.[2] The alternative suggests that local Ca^{2+} signaling microdomains, secluded from the contractile oscillations, feed into specific signaling cascades, such as calcineurin activation.[3,4] In this issue of *Circulation Research*, Tandan et al[5] propose that calcineurin binds directly to the sarcolemmal $Ca_V1.2$ voltage-gated Ca^{2+} channel and thereby modulates its function. By localizing calcineurin at the site of the major source of Ca^{2+} entry, this report could provide more support for the Ca^{2+}-signaling microdomain hypothesis and offer a new wrinkle into the understanding of hypertrophic signaling.

We already knew that the $Ca_V1.2$ Ca^{2+} channel and calcineurin could reside in the same neighborhood. In neurons, for example, the A-kinase anchoring protein AKAP79/150 has been shown to act as a scaffold for both the $Ca_V1.2$ channel and calcineurin.[6] $Ca_V1.2$ also functions as part of a complex with PKCα and calcineurin in vascular smooth muscle cells; here, AKAP79/150 is also thought to be the scaffold.[7] In both cases, the coupled calcineurin activates NFAT via Ca^{2+} influx through $Ca_V1.2$. Now, Tandan et al[5] report that in cardiac myocytes, calcineurin and $Ca_V1.2$ can shed their AKAP chaperone and cohabit. They identify direct calcineurin-binding sites on the $Ca_V1.2$ N and C termini. Nevertheless, $Ca_V1.2$ is a strange bedfellow. Both calcineurin-binding sites (the most extensively characterized in the distal C terminus [amino acids 1943 to 1971] and another, unidentified site in the N terminus) lack a consensus VIVIT-like motif found in many validated calcineurin-binding proteins, such as NFAT[8] and AKAP79/150.[9] Tandan et al also report that the interaction with the $Ca_V1.2$ C terminus renders the channel a calcineurin substrate and that calcineurin reverses PKC phosphorylation. Thus, this report may define a new calcineurin interaction motif and a novel mode of action.

A particularly notable finding is that calcineurin positively regulates $Ca_V1.2$ in neonatal myocytes. Although adenoviral overexpression of calcineurin increased $Ca_V1.2$ currents,[5] consistent with previous reports that calcineurin-induced hypertrophy augmented $Ca_V1.2$ currents in myocytes,[10,11] none of these experiments distinguished whether calcineurin directly potentiated the $Ca_V1.2$ currents or if the increased current amplitude was an indirect outcome of the resulting cellular hypertrophy. Here, Tandan et al[5] report that calcineurin inhibition (via cyclosporin) induced an immediate and partially reversible potentiation of $Ca_V1.2$ currents in neonatal myocytes. The rapidity and reversibility of these effects suggest a direct effect of calcineurin on $Ca_V1.2$ rather than transcriptional regulation via actions on NFAT. The potentiating effects of calcineurin on $Ca_V1.2$ in neonatal myocytes are surprising, however, because a recent study suggests that the predominant effect of phosphorylation on cardiac $Ca_V1.2$ by PKC, which calcineurin is proposed to antagonize, is potentiation.[12] This mechanism of action for calcineurin also provides a stark contrast to that reported in neurons,[6] where AKAP79/150-bound calcineurin opposes potentiation of $Ca_V1.2$ by PKA. In neonatal myocytes, Tandan et al found that neither adenoviral overexpression of calcineurin nor RCAN1 (a calcineurin inhibitor) affected isoproterenol-stimulated increase in $Ca_V1.2$ current amplitude. Thus, the specific means by which calcineurin produces this intriguing rapid and reversible $Ca_V1.2$ potentiation are not yet apparent.

So, does this $Ca_V1.2$-bound calcineurin with newly defined properties activate NFAT, as the AKAP-bound calcineurin

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(*Circ Res*. 2009;105:7-8.)

***Circulation Research* is available at http://circres.ahajournals.org**
DOI: 10.1161/CIRCRESAHA.109.201236

does in neurons,[6] and does the channel-phosphatase complex participate in the hypertrophic signaling pathway? The proximity of this fundamental Ca^{2+}-sensitive signaling molecule to the chief source of Ca^{2+} entry makes this an exciting possibility, but we will have to wait for follow-up studies to test this hypothesis. Although overexpression of calcineurin induced hypertrophy, as shown previously,[1] the authors have not yet established whether induction of hypertrophy depends on direct binding of calcineurin to $Ca_V1.2$. Specific studies designed to test whether calcineurin-induced hypertrophy and hypertrophy-induced NFAT activation are altered when the channel–phosphatase interaction has been disrupted will be necessary to resolve this issue. Other questions arising from this report include whether any of the intriguing potentiating effects of calcineurin on $Ca_V1.2$ Ca^{2+} current result from the direct binding between the two or derive from actions of another pool calcineurin untethered from the channel. Also unexplored is whether the observed $Ca_V1.2$ current potentiation is a result of calcineurin-mediated dephosphorylation of $Ca_V1.2$ or of another, intermediate substrate. Future studies will tell whether the calcineurin–$Ca_V1.2$ coupling proves a fruitful partnership.

Sources of Funding

Supported by National Institutes of Health grants HL088089 and HL071165. G.S.P. is an Established Investigator of the American Heart Association (Grant 0740030N).

Disclosures

None.

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KEY WORDS: calcineurin ■ L-type Ca^{2+} channel ■ hypertrophy ■ phosphatase

Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

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doi: 10.1161/CIRCRESAHA.109.201236
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571