NO generation by β-AR stimulation to activate CaMKII

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This editorial refers to ‘NO-dependent CaMKII activation during β-adrenergic stimulation of cardiac muscle’ by D.A. Gutierrez et al., pp. 392–401, this issue.

Ca2+-Calmodulin (CaM) kinase II (CaMKII) activation depends predominantly on modifications of amino acids within the regulatory domain on this multidomain kinase. A number of mechanisms of activation have been described since the original mode of activation, via Ca2+-CaM binding and phosphorylation of a threonine residue in the regulatory domain of CaMKII, was demonstrated. Gutierrez et al.1 propose a mechanism of CaMKII activation by nitrosation.

CaMKII serves as a link between β-adrenergic stimuli with subsequent changes in Ca2+ levels to the cellular response in cardiomyocytes. CaMKII phosphorylates dozens of substrates in many cell types. When activated by binding of Ca2+-CaM, CaMKII subunits can autophosphorylate neighboring subunits at Thr-287 (Thr287) within the CaMKII holoenzyme.2 Autophosphorylation at Thr287 results in autonomous kinase activity, converting to an activation state independent of Ca2+-CaM binding. A number of studies have shown that CaMKII can achieve sustained activity even in the absence of phosphorylation at Thr287. Mechanisms to attain CaMKII sustained include substrate binding,1,3–6 oxidation of the paired methionine residues (281/282) proximal to the Thr287,7 and now nitrosation of cysteine residues.1 Modifications that activate CaMKII occur primarily in the regulatory domain (Figure 1). These events prevent the catalytic domain from binding to the regulatory domain and allow CaMKII to retain catalytic activity for prolonged times after Ca2+ concentrations subside. Full CaMKII activity is observed with Thr287 phosphorylation and Ca2+-CaM binding while these other mechanisms of CaMKII activation show reduced CaMKII activity (for review, see Coultrap and Bayer8).

β-Adrenergic receptor (AR) stimulation of CaMKII activation was first linked to cardiac excitation–contraction coupling by Wang et al.9 later Ogrodnik and Niggli10 proposed the increase in sarcoplasmic reticulum (SR) Ca2+ release by β-AR stimulation lead to individual cardiac SR Ca2+ release events (sparks) via CaMKII activation. In their study, Gutierrez et al.11 show that a PKA-independent phosphorylation of the ryanodine receptor (Ryr) by β-AR signalling caused an increase diastolic Ca2+ spark frequency. This increase in spark frequency required CaMKII activation and is an indicator of spontaneous Ca2+ release from the SR. The spontaneous SR Ca2+ release occurs with increased frequency with β-AR signalling12 during diastole, the relaxation phase of the heartbeat. In the current study, the increase in spark frequency was blocked by the addition of either CaMKII or nitric oxide (NO) inhibitors and was not affected by reactive oxygen species (ROS) scavengers; implying that the β-AR stimulation increased NO-dependent activation of CaMKII; a novel mode of CaMKII activation.

NO is a short-lived redox molecule synthesized from l-arginine by the catalytic reaction of NO synthases. NO mediates diverse physiological functions including neurotransmission, regulation of vascular tone, cellular communication, inflammation, and immune responses. The cardiac NO synthases are constitutively expressed and their enzyme activity is Ca2+ dependent.12 Post-translational modification by NO can modify a number of amino acid residues, including nitrosation of thiol and amine groups. In this study, the authors show post-translational modification of CaMKII by nitrosation of cysteine residues (Figure 1). They use a series of experiments to test whether β-AR stimulation of ventricular cardiomyocytes increases Ca2+ sparks results from CaMKII activation by NO. First, they detected an increase in NO after isoproterenol (ISO) application in isolated cardiomyocytes. Applying the NO donor, S-nitroso glutathione to the cells, could increase Ca2+ spark frequency similar to ISO treatment. They then show that adding an inhibitor of CaMKII suppressed the effect of applying GSNO, implying the NO-stimulated increase in Ca2+ sparks occurs via CaMKII activity. Although these results only indirectly show activation of CaMKII by an increase in NO, they further show that CaMKII activity can be induced in vitro by NO and show CaMKII immuno-reactivity with an antibody that recognizes S-nitroso cysteine residues. The level of CaMKII nitrosation was increased ~30% with GSNO, which was reflected by a more than two-fold increase in CaMKII activity with GSNO application. A similar level of CaMKII nitrosation was shown for ROS and NO (~10–20% of full activity which occurs when Thr287 is phosphorylated and Ca2+-CaM bound).1,8 Taken together, their work shows NO activity is generated in cardiomyocytes by β-AR stimulation during diastole and this increase in NO activates CaMKII. The authors propose that
up to three cysteine residues in the catalytic and regulatory domains of CaMKII may be nitrosated. More conclusive evidence of the NO mode of CaMKII activation will come with mutagenic studies of CaMKII. Mutating each or a combination of these three proposed cysteine residues to neutral amino acids should block the increase of NO-activated Ca\(^{2+}\) sparks, under conditions tested.

β-AR treatment was used to test the idea that cross-talk between NO and Ca\(^{2+}\) signalling pathways contributes to arrhythmogenic diastolic Ca\(^{2+}\) release and Ca\(^{2+}\) waves during adrenergic stress. ISO treatment increased the frequency of Ca\(^{2+}\) sparks within minutes after stimulation, without a change in SR Ca\(^{2+}\) content. Field stimulation of isolated cardiomyocytes showed similar increases in NO-mediated Ca\(^{2+}\) frequency and spontaneous waves from CaMKII-activation of Ryrs. SR Ca\(^{2+}\) content was not a factor in these SR Ca\(^{2+}\) release events. Together these data indicate a central role of Ryrs in Ca\(^{2+}\) release events activated by nitrosated CaMKII, but without a requirement for SR Ca\(^{2+}\) uptake. Whether other NO-inducing effects in the cell will also activate CaMKII by nitrosation remains to be tested.

**Future directions**

What further activating amino acids that are modified within the CaMKII regulatory domain have yet to be discovered? Most recently, Erickson et al.\(^1\) show CaMKII activation by O-linked N-acetyl glucosamine modification at CaMKII serine-280. Modification of residues conserved among CaMKII isoforms and among paralogues in different species and therefore thought to have functional value, includes histidine-283 and other amino acids that may be phosphorylated and perhaps acetylation of lysine-299 (Figure 1) may be discovered as a regulatory modification.

**Conflict of interest:** none declared.

**References**


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**Figure 1** Domains of the CaMKII tripartite enzyme. Posttranslational modifications in the regulatory domain provide multiple sites of regulation. Examples of amino acid residues, conserved among the different forms of CaMKII, that are possible sites for modification as predicted by peptide scanners, such as biocuckoo.org.