VIEWPOINT

CaMKII regulation of phospholamban and SR Ca^{2+} load

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Introduction

During cardiac action potential, Ca^{2+}enters the cell through the L-type Ca^{2+} channels to trigger Ca^{2+} release from the sarcoplasmic reticulum (SR), which activates the myofilaments to drive contraction. The decrease in cytosolic Ca^{2+} leads to relaxation. This decrease is mainly induced by sarco(endo)plasmic reticulum Ca^{2+}-ATPase, isoform 2a (SERCA2a), which mediates Ca^{2+} uptake into the SR, and to a lesser extent by the Na^{+}/Ca^{2+} exchanger (NCX), which transfers Ca^{2+} to the extracellular space. By mediating SR Ca^{2+} uptake, the activity of SERCA2a also influences cardiac contractility, since it determines the size of the luminal Ca^{2+} store that is available for release in the next beat. The activity of SERCA2a, which in humans determines the rate of removal of >70% of cytosolic Ca^{2+}, is under the control of the closely associated SR protein phospholamban (PLN), a small phosphoprotein of 52 amino acids. Dephosphorylated PLN inhibits the affinity of SERCA2a for Ca^{2+}, and PLN phosphorylation relieves this inhibition.

The use of gene knockout and transgenic mouse models, in which the expression levels of PLN have been altered, constituted a crucial step in the recognition of the role of PLN in the regulation of myocardial performance. Ablation of PLN produced enhanced contractility and relaxation.[1] This hypercontractile function of PLN-deficient hearts ($PLN^{-}/^{-}$) was associated with increases in the affinity of SERCA2a for Ca^{2+} and in SR Ca^{2+} content. Opposite results were obtained in mice with PLN overexpression. In addition to the PLN expression levels, SERCA2a activity is also regulated by PLN phosphorylation. There are two PLN phosphorylation sites that are physiologically relevant: Ser^{16} residue, phosphorylated by protein kinase A (PKA); and Thr^{17}, phosphorylated by Ca^{2+}-calmodulin–dependent protein kinase (CaMKII). Phosphorylation of these sites reverses the inhibition of SERCA2a by PLN, thus increasing the affinity of the enzyme for Ca^{2+} and the rate of SR Ca^{2+} uptake. This in turn leads to increases in SR Ca^{2+} load, SR Ca^{2+} release, and myocardial contractility. The status of PLN phosphorylation also depends on the activity of the type 1 phosphatase, the major SR phosphatase, which specifically dephosphorylates PLN.

CaMKII-dependent PLN phosphorylation in physiological situations: β-adrenergic stimulation

Cardiac function is regulated on a beat-to-beat basis through the sympathetic nervous system. β1-adrenergic receptor stimulation (β-ARS) induces positive chronotropic, inotropic, and relaxant effects—the so-called fight or flight response—which is considered the most effective mechanism to acutely increase cardiac output. Activation of β-ARS by β1-agonists at the cell membrane initiates a signal-transduction pathway that proceeds through Gs proteins to stimulate cyclic adenosine monophosphate (cAMP) formation by adenylate cyclase and PKA activation. PKA then phosphorylates and alters the function of several cardiac proteins, among which PLN is predominant in determining the relaxant and inotropic effects of β-agonists[1] by increasing SR Ca^{2+} uptake and load (Figure 1).

Although β-ARS results in PLN phosphorylation at Ser^{16} (PKA site) and Thr^{17} (CaMKII site), the relevance of Thr^{17} phosphorylation in the relaxant and inotropic effects of β1-agonists has remained largely equivocal. Experiments in transgenic mice, expressing either wild-type PLN or the Ser^{16}→Ala mutant PLN, demonstrated that the phosphorylation of Ser^{16} of PLN is a prerequisite for the phosphorylation of Thr^{17}. As will be discussed, phosphorylation of Ser^{16} may be required to enhance cytosolic $Ca^{2+}$to the necessary level for CaMKII activation and Thr^{17} phosphorylation.

Experiments in Thr^{17}→Ala mutant PLN hearts further showed that phosphorylation of Ser^{16} was sufficient for mediating the maximal cardiac responses to β-ARS. More recent studies demonstrated that transgenic mice expressing

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ABBREVIATIONS **β-ARS** = β1-adrenergic receptor stimulation; **CaMKII** = Ca^{2+}-calmodulin–dependent protein kinase; **cAMP** = cyclic adenosine monophosphate; **Epac** = exchange protein activated by cAMP; **I/R** = ischemia/reperfusion; **NCX** = Na^{+}/Ca^{2+} exchanger; **PKA** = protein kinase A; **PLN** = phospholamban; **RyR2** = ryanodine receptors type 2; **SERCA2a** = sarco(endo)plasmic reticulum Ca^{2+}-ATPase, isoform 2a; **SR** = sarcoplasmic reticulum (Heart Rhythm 2011;8:784–787)

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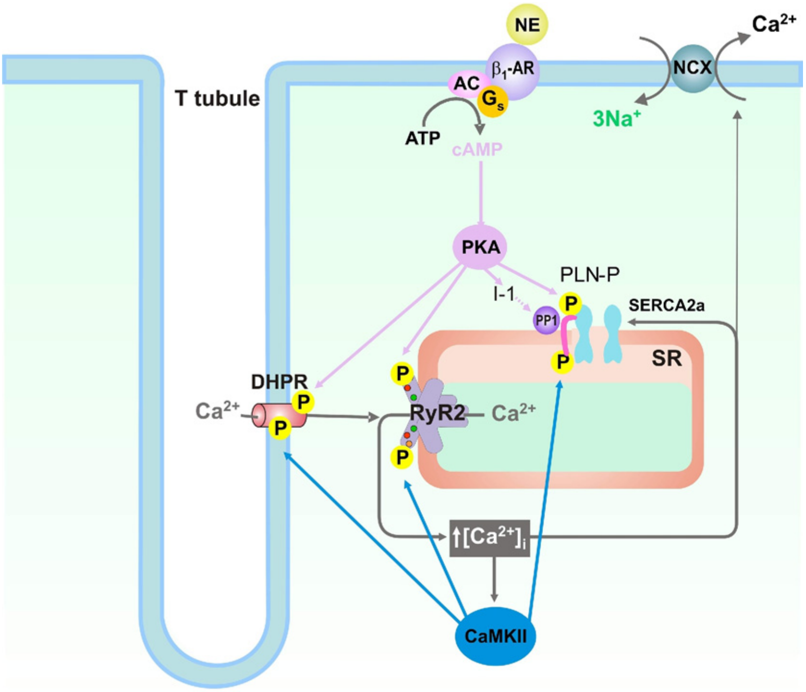

Figure 1 Schematic representation of cAMP/PKA/CaMKII cascades triggered by β-ARS. β-ARS leads to increases in cAMP and PKA. PKA-dependent phosphorylation of different proteins involved in Ca^{2+} handling increases intracellular Ca^{2+}. The increase in intracellular Ca^{2+} would favor CaMKII activation and CaMKII-dependent phosphorylation of various targets like the Thr^{17} site of PLN. PKA activation also inhibits type 1 phosphatase, the major phosphatase that dephosphorylates PLN. This inhibition would contribute to maintain both PKA and CaMKII-dependent phosphorylations.

a CaMKII inhibitory peptide targeted to the longitudinal SR (AIP4-LSR TG) exhibit reduced PLN Thr^{17} phosphorylation, decreased SR Ca^{2+} uptake, prolonged twitch Ca^{2+} transient decline, and a decrease in basal contraction and relaxation rates. However, the response to isoproterenol remained unaltered. Similarly, although SR Ca^{2+} content was significantly reduced in cardiomyocytes from another genetic model of cardiac CaMKII inhibition (AC3-I mice), these cells exhibited normal physiological responses to acute isoproterenol application.[2] These findings suggested either a predominant role of the phosphorylation of Ser^{16} over that of Thr^{17} in the mechanical effect produced by β-ARS or that cardiomyocytes can successfully compensate for Thr^{17} mutation and/or CaMKII inhibition.

Supporting the first possibility, kinetic experiments comparing phosphorylation of the Ser^{16} and Thr^{17} sites of PLN showed a correlation between contractility and cAMP elevation as well as phosphorylation of the PKA site of PLN but not of the CaMKII site of PLN during acute β-ARS. However, experiments that combined phosphorylation site-specific antibodies with quantification of ^{32}P incorporation into PLN in intact hearts indicated that phosphorylation of Thr^{17} accounted for approximately 50% of total PLN phosphorylation and enhancement of the relaxation rate at high isoproterenol concentrations (≥10 nM). In these experiments, no contribution of CaMKII to PLN phosphorylation could be detected at the lower isoproterenol doses.[3] In line with these findings, other experiments demonstrated that the dose-response curve of Thr^{17} phosphorylation to isoproterenol was shifted to the right, compared with that of Ser^{16} phosphorylation, clearly indicating that Ser^{16} was the only phosphorylated site at the lowest isoproterenol concentrations. These results might explain the failure to find significant PLN phosphorylation in the Ser^{16}→Ala mutant PLN mice, since the lack of phosphorylation of Ser^{16} would preclude the increase in intracellular Ca^{2+} necessary to phosphorylate Thr^{17} (Figure 1). Similarly, they might also provide a clue to interpreting results of experiments performed with relatively low extracellular Ca^{2+}, in which the contribution of Thr^{17} to total PLN phosphorylation was much lower than that observed in isolated rat hearts labeled with ^{32}P[3].

Experiments using the PKA inhibitor H-89 further confirmed that activation of PKA is required for β-AR-mediated phosphorylation of the Thr^{17} site. Taken together, these findings would support the idea that CaMKII is a β-AR mediator, with PKA as its upstream activator through the increase in intracellular Ca^{2+}. Interestingly, sustained β-ARS enhanced cell contraction and Ca^{2+} transients by a mechanism that is largely PKA independent but sensitive to CaMKII-inhibitors, underscoring the role of CaMKII during β-ARS under these conditions.

In addition, β-ARS activates the cAMP-binding protein Epac, independently of PKA. Activation of Epac has been shown to increase CaMKII activity and phosphorylation of Thr^{17} of PLN. However, the consequences of Epac-dependent Thr^{17} phosphorylation remain unclear since Epac has been shown to either increase or decrease Ca^{2+} transients. These apparently disparate results may arise from Epac-dependent effects on other proteins involved in Ca^{2+} handling, since Epac activation also produces SR Ca^{2+} leak.