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CAMKII REGULATION OF PHOSPHOLAMBAN AND SR Ca^{2+} LOAD

Alicia Mattiazzi, M.D.[1] and **Evangelia G. Kranias, Ph.D.**[2,*]

[1]Centro de Investigaciones Cardiovasculares (UNLP-Conicet), Facultad de Ciencias Médicas, 60 y 120, (1900) La Plata, Argentina

[2]Department of Pharmacology, University of Cincinnati College of Medicine, 231 Albert Sabin Way, Cincinnati, Ohio 45267

Abstract

Phospholamban (PLN) is a small phosphoprotein in the cardiac sarcoplasmic reticulum (SR). Dephosphorylated PLN tonically inhibits the SR Ca^{2+}-ATPase (SERCA2a) and phosphorylation of PLN, at either Ser^{16} by PKA or Thr^{17} by Ca^{2+}-calmodulin-dependent protein kinase (CaMKII), reverses this inhibition. Consequently, there are increases in SERCA2a activity, SR Ca^{2+} uptake rate and SR Ca^{2+} load. Through this mechanism, PLN is a major regulator of basal cardiac Ca^{2+} cycling, contractility and relaxation and the main determinant of β-adrenergic mechanical responses in the heart. In this article, we briefly review the functional role of CaMKII-dependent PLN phosphorylation at Thr^{17} site and the new findings that link this phosphorylation to beneficial or detrimental effects in pathophysiological situations.

Keywords

CaMKII; Phospholamban phosphorylation; Sarcoplasmic reticulum; Ryanodine receptors; Acidosis; Ischemia/reperfusion

INTRODUCTION

During cardiac action potential, Ca^{2+} enters the cell through the L-type Ca^{2+} channels to trigger Ca^{2+} release from the SR, which activates the myofilaments to drive contraction. The decrease in cytosolic Ca^{2+} leads to relaxation. This decrease is mainly induced by 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.

*Corresponding Author tel: 513-558-2327; fax: 513-558-0646; litsa.kranias@uc.edu.

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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 relaxation1. 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 PKA and Thr^{17}, phosphorylated by 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 (PP1), 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-AR by β1-agonists at the cell membrane, initiates a signal-transduction pathway that proceeds through Gs proteins to stimulate cyclic AMP (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-agonists1, by increasing SR Ca^{2+} uptake and load (Figure 1).

Although β-AR-stimulation 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 Ser16→Ala mutant PLN, demonstrated that the phosphorylation of Ser^{16} of PLN is a prerequisite for the phosphorylation of Thr^{17}. As will be further discussed below, 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 Thr17→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 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 application2. 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 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 which 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 doses3. 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 to 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 Ser16→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 in 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}P3. 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 which 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 phosphoryltion 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. Unfortunately, a detailed analysis of the effects of Epac on SR Ca^{2+} uptake and load is still lacking.

CaMKII-dependent phosphorylation of PLN in pathological situations

Recent studies showed the involvement of CaMKII-dependent PLN phosphorylation in different pathophysiological situations. Interestingly, CaMKII-dependent phosphorylation of PLN and the consequent increase in SR Ca^{2+} uptake, have beneficial effects for heart performance in some cases, whereas they are detrimental in others. Obviously, in all situations the effects of CaMKII-dependent PLN phosphorylation is the same, i.e. to increase SR Ca^{2+} refilling. As will be discussed below, the extent of SR Ca^{2+} load produced by PLN phosphorylation as well as the status/characteristics/activity of other proteins [ryanodine receptors (RyR2) and NCX], which share with PLN the task of regulating intracellular Ca^{2+} handling, may be key determinants of whether CaMKII-dependent PLN phosphorylation and the resultant increase in SR- Ca^{2+} load, evolves towards a final beneficial or detrimental outcome.

Acidosis—Intracellular acidosis is associated with a decrease in the ability of the heart to generate tension, which is largely due to a decrease in myofilament Ca^{2+} responsiveness. This initial impairment in cardiac performance is followed by a spontaneous recovery, which requires an intact SR and has been shown to be dependent on the activity of CaMKII. It was further demonstrated that phosphorylation of the Thr^{17} site of PLN transiently increased at the onset of acidosis and is responsible for the increase in SR Ca^{2+} load and most of the mechanical recovery that follows the acidotic insult. This CaMKII phosphorylation of PLN would thus provide a mechanism to overcome the direct depressant effect of acidosis on the Ca^{2+} pump. Interestingly, upon returning to normal pH, the CaMKII-dependent increase in SR Ca^{2+} load during acidosis produces SR Ca^{2+} leak, (promoted by the relief of the acidosis-induced inhibition of RyR2 when the pH is restored to normal), followed by Ca^{2+} efflux and Na^{+} influx through the NCX. This provides the

molecular bases for the CaMKII-dependent ventricular arrhythmias, observed when pH is normalized after acidosis. Thus, the increase in SR Ca^{2+} load produced by CaMKII-dependent PLN phosphorylation during acidosis, is responsible for apparently opposite outcomes: beneficial, since it favors Ca^{2+} handling and mechanical recovery, and detrimental, because it puts the heart at risk of arrhythmias.

Ischemia/reperfusion (I/R)—In the last few years, a dual effect of CaMKII-dependent protein phosphorylation (beneficial and detrimental) has been described in the scenario of I/R in the intact heart. The beneficial effect of CaMKII occurs in the stunned heart, a fully reversible post-ischemic dysfunction and is due to an increase in the phosphorylation of the Thr^{17} site of PLN that takes place at the onset of reperfusion. Experiments in transgenic mice in which Thr^{17} and/or Ser^{16} sites of PLN were mutated to Ala and direct measurements of intracellular Ca^{2+} were performed, demonstrated that this phosphorylation was essential for the recovery of Ca^{2+} transients and contractility in the stunned heart during reperfusion. The detrimental action of CaMKII takes place during infarction, a post-ischemic irreversible cardiac injury in which cardiac cells die by necrosis and apoptosis. It has been shown that CaMKII-dependent phosphorylation of PLN at the onset of reperfusion is not only unsuccessful in rescuing cardiac myocytes from death, but appears as part of the deleterious cascade mediated by CaMKII responsible for apoptosis and necrosis4. Interestingly, these deleterious effects appear to be associated with a degradation of RyR2 and an increase in SR Ca^{2+} release. Similarly opposite results regarding the final outcome of PLN phosphorylation and increase in SR Ca^{2+} uptake on myocardial performance, were described in a variety of experimental conditions. For example, the protective effect (decreased number of apoptotic cells) of chronic CaMKII inhibition in transgenic hearts expressing a CaMKII inhibitory peptide (AC3-I) and submitted to myocardial infarction, was absent in the cross mice of AC3-I overexpression and PLN ablation. In these animals, the absence of the inhibitory effect of PLN greatly enhanced SR Ca^{2+} uptake and load5. In contrast, other results suggest that increased PLN phosphorylation observed after inducible expression of inhibitor-1 has beneficial effects in I/R injury.

CONCLUSIONS

The results summarized above indicate that increases in SR Ca^{2+} uptake by Thr^{17} phosphorylation of PLN may contribute to the mechanical effects of acute β-ARs and are responsible for the sustained actions of β-1 agonists in the intact heart. Moreover, CaMKII-dependent PLN phosphorylation may paradoxically produce either favorable or harmful cardiac effects. Since the sole effect of PLN phosphorylation is to increase SR Ca^{2+} uptake and since SR Ca^{2+} content is the net result of SR Ca^{2+} uptake and release processes, one can hypothesize that the progression towards a beneficial or detrimental effect of CaMKII activation and PLN phosphorylation depends on two main factors: 1) The extent of SR Ca^{2+} reuptake; and 2) The status/characteristics of other proteins that are also involved in SR Ca^{2+} handling, among which the RyR2 is the main candidate (Figure 2). A moderate or even high increase in SR Ca^{2+} uptake (and content) due to PLN phosphorylation, would enhance RyR2 opening due to the regulatory effect of intra-SR Ca^{2+}. However, in the absence of additional RyR2 modifications, the increase in SERCa2a activity produced by PLN phosphorylation may still cope with the enhanced diastolic SR Ca^{2+} release and improve SR Ca^{2+} handling. In contrast, even moderate increases in SR Ca^{2+} content may increase diastolic SR Ca^{2+} release under conditions where the RyR2 activity is altered independently of intra-SR Ca^{2+}-induced modifications, enhancing the propensity to arrhythmias and leading to mitochondrial Ca^{2+} overload. This would favor apoptosis and eventually necrosis. Of interest, a similar situation to the one depicted in Figure 2B has been recently suggested to take place when the dilated cardiomyopathy that occurred in mice with CaMKIIδC overexpression could not be rescued by crossing with PLN−/− mice, in an attempt to

attenuate PLN inhibitory effects on SERCA2a and ameliorate Ca^{2+} handling. Thus, increasing SERCA2a activity by PLN phosphorylation has indeed the potential of producing salutary effects in a number of diseases. However, these effects may be achieved only under certain conditions in which diastolic Ca^{2+} release could be satisfactory controlled.

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Abbreviations

PLN	Phospholamban
SR	Sarcoplasmic Reticulum
SERCA2a	Sarco(endo)plasmic Reticulum Ca^{2+}-ATPase, isoform 2a
CaMKII	Ca^{2+}-calmodulin-dependent protein kinase
PKA	Protein Kinase A
NCX	Na^{+}/Ca^{2+} exchanger
PP1	type 1 phosphatase
β-ARs	β1-adrenergic receptor stimulation
cAMP	cyclic adenosine monophosphate
Epac	exchange protein activated by cAMP
RyR2	Ryanodine Receptors type 2
I/R	Ischemia/reperfusion.

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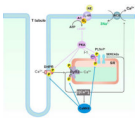

Figure 1.
Schematic representation of cAMP/PKA/CaMKII cascades triggered by βAR-stimulation. β-ARs leads to increases in cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA). PKA-dependent phosphorylation of different proteins involved in Ca^{2+} handling increases intracellular Ca^{2+}. 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 PP1, the major phosphatase that dephosphorylates PLN. This inhibition would contribute to maintain both PKA and CaMKII-dependent phosphorylations.

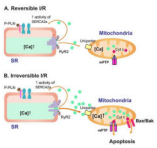

Figure 2.
Hypothetical explanation of the dual effects of CaMKII in ischemia/reperfusion (See text for details).