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β-Adrenergic receptor signaling in the heart: Role of CaMKII

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Abstract

The multifunctional Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) targets a number of Ca^{2+} homeostatic proteins and regulates gene transcription. Many of the substrates phosphorylated by CaMKII are also substrates for protein kinase A (PKA), the best known downstream effector of β-adrenergic receptor (β-AR) signaling. While PKA and CaMKII are conventionally considered to transduce signals through separate pathways, there is a body of evidence suggesting that CaMKII is activated in response to β-AR stimulation and that some of the downstream effects of β-AR stimulation are actually mediated by CaMKII. The signaling pathway through which β-AR stimulation activates CaMKII, in parallel with or downstream of PKA, is not well-defined. This review considers the evidence for and mechanisms by which CaMKII is activated in response to β-AR stimulation. In addition the potential role of CaMKII in β-AR regulation of cardiac function is considered. Notably, although many CaMKII targets (e.g., phospholamban or the ryanodine receptor) are central to the regulation of Ca^{2+} handling, and effects of CaMKII on Ca^{2+} handling are detectable, inhibition or gene deletion of CaMKII has relatively little effect on the acute physiological contractile response to β-AR. On the other hand CaMKII expression and activity are increased in heart failure, a pathophysiological condition characterized by chronic stimulation of cardiac β-ARs. Blockade of β-ARs is an accepted therapy for treatment of chronic heart failure although the rationale for its beneficial effects in cardiomyocytes is uncertain. There is growing evidence that inhibition or gene deletion of CaMKII also has a significant beneficial impact on the development of heart failure. The possibility that excessive β-AR stimulation is detrimental because of its effects on CaMKII mediated Ca^{2+} handling disturbances (e.g., ryanodine receptor phosphorylation and diastolic SR Ca^{2+} leak) is an intriguing hypothesis that merits future consideration.

Keywords

CaMKII; Beta-adrenergic receptor signaling; Excitation–contraction coupling; Heart failure; Hypertrophy; Apoptosis; Arrhythmia

1. Introduction

Sympathetic stimulation of cardiac β_1-adrenergic receptors (β-AR) induces positive inotropic and chronotropic effects–the so-called "fight or flight response," the most effective mechanism to acutely increase output of the heart. Cyclic AMP (cAMP) formed through β-AR mediated activation of adenylyl cyclase (AC), and the subsequent activation of its downstream target, the cAMP dependent protein kinase (PKA), are well-described mediators with targets that promote maximal myocardial performance. Excessive sympathetic nervous system activity observed in heart failure can cause detrimental effects such as cardiomyocyte death [1–3], and β-AR blockers are one of the standard therapeutic approaches for the treatment of chronic heart

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failure. In many ways, however, the rationale for the salutary effects of β-AR blockade in heart failure treatment appears paradoxical since these agents further reduce contractile performance by reducing inotropic and chronotropic effects of catecholamines. Promising new therapeutic strategies for heart failure will most likely result from better understanding of downstream effectors of β-AR signaling which allow one to separate beneficial from detrimental pathways. For example, stimulation of β-AR activates the two most abundant AC types 5 and 6 in the heart to produce cAMP, but they appear to have opposite effects, with AC5 activity being detrimental [4] and AC6 being beneficial [5] for heart function. Other studies have suggested that downregulation of β-AR in heart failure–generally thought to be an adaptive and protective mechanism and one that should in theory mimic β-AR blockade–is maladaptive [6,7]. Inhibition of myocardial β-AR desensitization, using *in vivo* intracoronary adenoviral-mediated gene delivery of a peptide inhibitor of β-AR kinase (βARK1), was shown to improve cardiac function and prevent the development of heart failure in rabbit hearts subjected to myocardial infarction [6] or reverse cardiac dysfunction [7], suggesting that enhancing rather than blocking β-AR signaling has salutary effects.

Interestingly and perhaps one reason for the discrepancies above, not all downstream elements of the β-AR pathway are targets of the well established β-AR/cAMP/PKA signaling cascade. More than a decade ago, Baltas et al. [8] published the unexpected and still relatively unappreciated finding that β-AR stimulation activates Ca^{2+}/calmodulin dependent protein kinase (CaMK) II in the intact beating heart. The authors' conclusion was based on measurement of CaMKII autophosphorylation and phosphorylation of the CaMKII target phospholamban (PLN) in Langendorff-perfused rat hearts. Subsequently a substantial body of literature using different systems and endpoints has documented the involvement of CaMKII in mediating effects of β-AR stimulation on phosphorylation of Ca^{2+} handling proteins [9–11], Ca^{2+} release from the sarcoplasmic reticulum [10,12], contractility [13], hypertrophic gene expression [14], and apoptosis [2]. In contrast to our extensive knowledge regarding molecular events in the β-AR/cAMP/PKA signaling cascade, the mechanism by which stimulation of the β-AR results in CaMKII activation and the role of CaMKII in β-AR are not generally appreciated and poorly understood. Here we review evidence that a significant component of β-AR signaling is mediated through CaMKII, suggest that this pathway is particularly prominent under pathophysiological conditions, and consider whether the beneficial effects of β-AR blockade in heart failure might be explained by attenuation of CaMKII signaling. Interestingly, the effect of β-blocker therapy on the expression or activity of CaMKII in chronic heart failure has never been explored.

2. Activation of CaMKII by β-AR stimulation

2.1. Assessment of CaMKII activity

Essential to the hypothesis that CaMKII mediates the effects of β-AR stimulation is demonstrating that CaMKII is activated in response to β-adrenergic stimulation (Fig. 1). It is possible to measure the enzymatic activity of CaMKII and activation state using specific substrates and addition of its regulators, $Ca^{2+}$and calmodulin, and this direct approach has in fact been used in some studies [2,15–18]. An alternative is to examine the autophosphorylation of the enzyme at Thr286–several antibodies to this site are commercially available– as this site is phosphorylated following Ca^{2+}/CaM binding to and associated with activation of CaMKII [19]. Much of the literature implicating CaMKII in β-AR action, however, has used more indirect readouts, specifically the phosphorylation of putative CaMKII specific target sites on Ca^{2+} regulatory proteins. The two best studied of these are Thr17 on phospholamban (PLN) and Ser2814 or Ser2815 (depending on the species) on the type 2 ryanodine receptor (RyR2). Notably both PLN and RyR2 also have nearby but distinct sites of phosphorylation by PKA (Ser16 on PLN, Ser2809 on RyR2). These latter sites are the accepted known targets of β-AR stimulation and play prominent roles in β-AR regulation of cardiac contraction and relaxation

by affecting Ca^{2+} reuptake by and release from the sarcoplasmic reticulum. More surprising is that the PLN (Thr17) and RyR2 (Ser2814) sites mentioned above which have been convincingly demonstrated to be CaMKII specific both *in vitro* [20] and *in vivo* [21] can also be phosphorylated following β-AR stimulation [21,22]. These data implicate CaMKII as a β-AR mediator, as discussed below.

2.2. Does the β-AR effect on Ca^{2+} mediate activation of CaMKII?

CaMKII activity responds to the frequency and magnitude of Ca^{2+} signals, both of which are increased by β-AR stimulation of the heart [23]. Inhibition of Ca^{2+} entry via L-type Ca^{2+} channel (LTCC) achieved by lowering Ca^{2+} or using the LTCC blocker nifedipine [9], or depletion of SR Ca^{2+} by ryanodine [24] abolish CaMKII target PLN Thr17 phosphorylation induced by β-AR stimulation. Conversely, activation of LTCC using the agonist Bay K8644 mimics the β-AR-stimulated PLN Thr17 phosphorylation [24]. In contrast, the PKA target PLN Ser16 is directly phosphorylated by PKA, independent of Ca^{2+} entry via LTCC [9,24]. It is not clear whether the normal sympathetic regulation of heart rate and contractility are paralleled by beat to beat changes in the activation of CaMKII. CaMKII activation has been modeled based on changes in activity of a FRET based Ca^{2+}-calmodulin (CaM) reporter [25]. The authors suggest, based on the kinetics of CaM activation, that phasic activation of CaMKII would occur only in response to high Ca^{2+} levels achieved "locally" in restricted compartments but not "globally" in the cytosol during excitation-contraction coupling [25, 26]. The function of such a specialized compartment could be fulfilled by the dyadic cleft where the local Ca^{2+} concentration (and CaM expression) is high as compared to the cytosol, because the LTCC and the RyR are located in close proximity. During β-adrenergic stimulation there are concomitant increases in Ca^{2+} entry via LTCC, SR Ca^{2+} release via RyR, and phosphatase inhibition. Together these would be expected to enhance dyadic cleft Ca^{2+} beyond the very low level maintained during normal beat-to-beat Ca^{2+} transients [26] and this could promote CaMKII activation. The nuclear envelope is another specialized compartment where CaMKII has been suggested to be activated locally by highly elevated Ca^{2+}–not directly by β-adrenergic activation but in response to $InsP_3$ signaling [27].

How dependent is CaMKII activation on local Ca^{2+} levels? As mentioned above, unlike the initial activation of CaMKII by Ca^{2+}/CaM, sustained activation occurs by autophosphorylation of CaMKII at Thr287 and this correlates with its downstream effects. Autophosphorylation of CaMKII is provoked by highly elevated Ca^{2+}. Interestingly, the same effect (i.e., autonomous CaMKII activity) can be elicited by ROS-induced oxidation of CaMKII at Met281/282, independent of autophophosphorylation [28]. The evidence that Ca^{2+}-independent, pro-oxidant conditions can activate CaMKII, and the extensive data showing that stimulation of β-AR can result in ROS production [29–31] suggest that ROS and not Ca^{2+} could link β-adrenergic signaling to CaMKII activity. However, the only study examining this possibility found that autophosphorylation rather than oxidation caused activation of CaMKII in response to β-AR stimulation [28].

2.3. Role of PKA in activation of CaMKII

The best-described effect of β-AR stimulation is mediated by the α subunit of the Gs protein, which activates adenylyl cyclase and subsequently increases PKA activity. Phosphorylation of PLN in response to β-AR stimulation in perfused rat hearts has been shown to occur initially through the effects of PKA at Ser16, followed by the effects of CaMKII at Thr17 [9]. Experiments using the PKA inhibitor H-89 in isolated perfused rat hearts provided further evidence that activation of PKA is also required for β-AR mediated phosphorylation of the Thr17 CaMKII site on PLN [11]. The same has been demonstrated for β-AR stimulated phosphorylation of the Ser2815 CaMKII site on RyR2 [10]. The increase in Ser2815 phosphorylation by isoproterenol is CaMKII dependent but probably also dependent on the

activation of PKA upstream of CaMKII, because inhibition of PKA and direct activation of PKA inhibited and mimicked the effect of β-AR stimulation, respectively [10]. These data support the concept that PKA is an upstream activator of CaMKII and suggest that CaMKII activation results from PKA-dependent increases in Ca^{2+}. There is also a more indirect mechanism by which PKA could stimulate CaMKII activity. Autonomous activity of CaMKII depends on its autophosphorylation at Thr286, which has in turn been suggested to be modulated by the phosphatase PP1 [32,33]. β-AR stimulation suppresses PP1 activity (via PKA-dependent phosphorylation of the PP1 inhibitor I-1 at Thr35.) The physiological relevance of this mechanism was *indicated* by the demonstration that phosphorylation of the CaMKII targets, RyR Ser2815 and PLN Thr17, in response to the β-AR agonist isoproterenol was reduced in hearts from I-1 KO mice [34].

PKA dependence of CaMKII activation in response to β-AR stimulation does not necessarily mean that targets of PKA and CaMKII are obligatorily phosphorylated in parallel. While CaMKII activation would be responsive to Ca^{2+} elevation and PPI activity, the localized expression of CaM and PP1 (and possibly other phosphatases) would ultimately regulate the kinetics and specificity of CaMKII target phosphorylation. Similarly PKA is found in signaling complexes with different A kinase anchoring proteins (AKAPs), phosphatases and phosphodiesterases, forming local signaling cascades [35,36]. Thus, β-AR stimulation may activate CaMKII via PKA, but phosphorylation of their respective targets could still be regulated relatively independent of each other.

2.4. PKA-independent β-adrenergic activation of CaMKII

In a study by Wang et al. [13], chronic β_1-AR stimulation of adult myocytes for 24 h was demonstrated to enhance the contraction amplitude to the same extend as acute β-AR stimulation. The major difference was, however, that acute effects were mediated via PKA whereas chronic effects were mediated via CaMKII (independent of PKA.) Both effects were reversed by a β-blocker or washout, suggesting that continued β-AR activation rather than autonomous CaMKII activity was responsible for the observed effects on contractility. What else might β-AR activation do that could elicit CaMKII mediated responses? It has recently become clear that PKA is not the only downstream effector of β-AR mediated elevation of cAMP. A guanine nucleotide exchange protein directly activated by cAMP (Epac) is a direct target for cAMP [37]. Several papers suggest that Epac can increase CaMKII activity in a PKA-independent manner in isolated adult rat cardiomyocytes, accompanied by enhanced CaMKII target phosphorylation at RyR2 Ser2815 and PLN Thr17 [18,38–40]. The Epac activator 8-CPT was shown to increase CaMKII autophosphorylation in two recent studies [18,39] and to increase myofilament Ca^{2+} sensitivity via CaMKII in adult ventricular myocytes [41]. Strikingly, Epac-mediated CaMKII activation showed the exact opposite effect on SR Ca^{2+} release in these studies, i.e., Epac activation either increased [39] or decreased Ca^{2+} transients [18,41]. The signaling pathway by which Epac activates CaMKII in parallel with and independently of PKA has not been fully delineated. Epac activates the Rap GTPase Rap2B and subsequently a newly described PLC isoform, PLCε [42]. Oestreich et al. [38,39] recently proposed a pathway by which Epac-mediated PLCε activation stimulates protein kinase C epsilon (PKCε) and hence CaMKII. The authors provide evidence that this pathway mediates β-AR-dependent regulation of SR Ca^{2+} release, in parallel with PKA, in cardiac myocytes. This β-AR and Epac mediated SR Ca^{2+} leak is CaMKII-dependent, but independent of PKA based on experiments in rabbit myocytes using inhibitors of CaMKII or PKA [12,43].

Catecholamine-induced enhancement of the diastolic SR Ca^{2+} leak can cause ventricular arrhythmia, and Epac has been shown to trigger ventricular arrhythmias through a mechanism dependent on CaMKII activity [44], supporting earlier studies that identified CaMKII as a proarrhythmic signaling molecule in the catecholamine-stimulated heart [45]. PKA-

independent activation of CaMKII via Epac may also play a role in the induction of myocardial hypertrophy resulting from chronic stimulation of β-ARs [14,16,40]. Knockdown of Epac1 [40] or inhibition of CaMKII suppresses the hypertrophic changes induced by β-AR stimulation in neonatal myocytes [14] and *in vivo* [16].

2.5. Is cAMP required for β-adrenergic CaMKII activation?

Activation of CaMKII through either PKA or through Epac would require cAMP produced by adenylyl cyclase. However, one study that focused on the regulation of the SR Ca^{2+} leak by β-AR stimulation and found that the leak was CaMKII-dependent also reported that it was independent of PKA and cAMP [12]. Direct stimulation of adenylyl cyclase using forskolin was found to stimulate the PKA-dependent Ca^{2+} transient amplitude but not the CaMKII-dependent SR Ca^{2+} leak [12]. That study suggests that an alternative, cAMP-independent pathway mediates β-adrenergic activation of CaMKII. What mechanism can explain cAMP-independent activation of CaMKII in response to β-AR stimulation? One possibility is that the α subunit of Gs, the heterotrimeric G protein that is coupled to the β-AR, directly activates the L-type Ca^{2+} channel independent of cAMP/PKA [46]. The βγ subunits do not appear to function in this way since inhibition of signaling through the βγ subunit of Gs does not prevent activation of CaMKII in response to β-AR stimulation [2]. In addition to the study mentioned above, CaMKII-dependent activation of the fetal gene program in response to chronic ISO was shown to be dependent on activating Ca^{2+} but independent of cAMP and PKA [14].

3. CaMKII targets in β-adrenergic signaling

Norepinephrine stimulates postsynaptic β-ARs in the heart resulting in positive chronotropic (heart beats faster), positive inotropic (heart beats stronger), and positive lusitropic (heart relaxes faster) response. Calcium plays a crucial role in regulating these β-adrenergic effects. As described above, Ca^{2+} enters the myocyte via the LTCC, which triggers the release of Ca^{2+} ions from the SR via calcium release channels (RyR). Re-uptake of Ca^{2+} into the SR occurs via SERCA and is regulated by PLN and this, along with extrusion of Ca^{2+} via NCX, completes the cardiac cycle. Stimulation of cyclic AMP production and activation of PKA facilitates the events described above at several steps. The possible role that CaMKII activation through the β-AR pathway plays in these responses bears consideration, especially because PKA and CaMKII share many intracellular target proteins including the LTCC, RyR and PLN.

3.1. Ryanodine receptor

RyR2 is the most abundant RyR isoform in the heart. Studies using mutated full-length RyR2 have indicated Ser2814 as the CaMKII and Ser2808 as the PKA phosphorylation sites, respectively [47,48]. A second major PKA phosphorylation site at Ser2030 on RyR2 has been suggested to respond to acute β-AR stimulation [49]. However, it appears that only very low amounts of phosphorylated Ser2030 are generated by stimulation with the β-AR agonist isoproterenol in quiescent rat cardiac myocytes [22]. Single-channel experiments and the analysis of the RyR2-S2808A knock-in mouse have indicated Ser2808 as the sole functional PKA phosphorylation site [50]. While it has been suggested by some studies that CaMKII can phosphorylate Ser2808 *in vitro* [51,52] and *in vivo* [53], this finding was not confirmed by others [22,48]. An early study by Witcher et al. demonstrated CaMKII-dependent phosphorylation of Ser2809 using a tryptic peptide sequence corresponding to residues 2807–2824 [52]. The phosphorylation was also shown using a rabbit antiserum made from a synthetic RyR peptide (residues 2805–2819). It is now clear that the tryptic peptide contained and the antiserum was likely directed against not only Ser2809 but also Ser2815. More than a decade later Wehrens et al. [48] demonstrated that full-length mutant RyR2 channels with an unphosphorylatable CaMKII (S2815A) site could be phosphorylated by PKA but not by CaMKII, implicating Ser2815 as the sole CaMKII site. Interestingly, phosphorylation of wild-

type RyR2 channels by exogenous PKA causes dissociation of the regulatory subunit FKBP12.6 from the channel (FKBP12.6 dissociates from Ser2809 phosphorylated RyR2), but this does not occur in response to CaMKIImediated phosphorylation [48]. Taken together, the findings suggest that Ser2809 and Ser2815 are specific sites for PKA and CaMKII, respectively, and that they have different functional consequences. In this regard the fact that stimulation of adult rat ventricular myocytes with isoproterenol can lead not only to the expected phosphorylation of Ser2808 by PKA but also to Ser2815 phosphorylation [22] suggests pleiotropic effects of β-AR signaling on RyR. RyR regulation by PKA and CaMKII has been reviewed recently [54].

3.2. Phospholamban

PLN can be directly phosphorylated by either PKA or CaMKII. *In vitro* assays showed that PKA exclusively catalyzes phosphorylation of PLN Ser16, whereas CaMKII gives exclusive phosphorylation of PLN Thr17 [20]. In the intact heart and in beating papillary muscle preparations, β-AR stimulation results in the phosphorylation of both Ser16 and Thr17 residues of PLN [21,55,56]. Recent data demonstrate that it is the β_1-AR subtype that mediates CaMKII activation and PLN Thr17 phosphorylation in response to isoproterenol infusion [57]. Time course experiments show that phosphorylation by PKA occurs within 30 s whereas CaMKII-mediated phosphorylation is seen within 1–3 min [21,24]. CaMKII-dependent phosphorylation at Thr17—in contrast to PKA-mediated phosphorylation at Ser16—is undetectable in quiescent myocytes stimulated with norepinephrine [32,58]. In the absence of Ca^{2+} there is Ser16 but no PLN Thr17 phosphorylation in response to β-adrenergic stimulation alone [59], because phosphorylation of Thr17 by CaMKII in response to β-AR stimulation requires influx of Ca^{2+} through the L-type Ca^{2+} channel [9] and release of Ca^{2+} from the SR [24]. It is possible, however, to modulate Thr17 (without affecting Ser16) by using the experimental compound Bay K8644 to increase LTCC activity [24]. In addition, while PKA-dependent PLN Thr17 phosphorylation has been shown in intact beating hearts [9–11], PKA does not directly phosphorylate Thr17, because SR-targeted inhibition of CaMKII in mouse hearts effectively blocks PLN Thr17 phosphorylation in response to isoproterenol [60,61]. The regulation of CaMKII-dependent PLN phosphorylation and its role under pathophysiological conditions is discussed in a recent review article [62].

3.3. Other CaMKII targets

L-type Ca^{2+} channel. It is well established that activation of PKA through β-adrenergic stimulation of cAMP production leads to enhanced Ca^{2+} current. The activity of the LTCC is regulated by β-adrenergic stimulation, as well as by Ca^{2+} itself, with a time course similar but not additive to that seen with β-adrenergic stimulation [63]. Ca^{2+}-dependent I_{Ca} facilitation is due to activation of CaMKII and phosphorylation of the LTCC [64–67]. It has been shown that CaMKII interaction with the pore-forming α_{1C} subunit of the L-type calcium channel is essential for Ca^{2+}-dependent facilitation of the LTCC [68,69]. CaMKII actions at the distal carboxyl-terminus of α_{1C} may also play a role in β-adrenergic augmentation of cardiac L-type Ca^{2+} current since α_{1C} (but not phosphorylation of the putative PKA site Ser1928 on α_{1C}) is required for this effect [70–72]. Recent data suggest that CaMKII also binds to the LTCC β_{2a} subunit where it phosphorylates the Thr498 site, facilitating I_{Ca} [73,74]. Interestingly, there are no data available that directly demonstrate CaMKII-dependent β-adrenergic modulation of Ca^{2+} channel activity. In fact, an earlier study from Anderson et al. demonstrated that CaMKII inhibition did not block I_{Ca} activation by β-AR stimulation [65]. Thus more experiments are needed to explore the role of LTCC in CaMKII-dependent β-adrenergic signaling.

Cardiac myosin binding protein-C (cMyBP-C), a component of the thick filament, plays a major role as both a structural and regulatory protein in the heart. Phosphorylation of cMyBP-C is important for normal heart function [75–77]. Hartzell and Glass demonstrated

phosphorylation of cMyBP-C by purified PKA and CaMKII *in vitro* [78]. Today three of the serines in cMyBP-C (Ser273, Ser282 and Ser302 in the mouse heart) are known to be substrates for PKA [79,80]. Ser282 can be phosphorylated by CaMKII in addition to PKA and this phosphorylation is thought to be a prerequisite for the phosphorylation of other sites [79]. The functional role of the distinct phosphorylation sites are not known. Cardiac MyBP-C becomes phosphorylated in response to acute β-AR stimulation [81,82]. Contrary to the effect of acute β-adrenergic stimulation, CaMKII-site phosphorylation in cardiac MyBP-C is decreased in patients with heart failure, hypertrophic cardiomyopathy and atrial fibrillation, conditions that are generally associated with increased activities of the sympathetic nervous system and CaMKII [83,84]. It is not known to date why cMyBP-C phosphorylation is paradoxically decreased under conditions of increased PKA and CaMKII signaling.

Class II histone deacetylases (HDACs) are known targets of CaMKII in the heart [27,85–89]. CaMKII phosphorylation of class II histone deacetylase 4 (HDAC4), a repressor of the MEF2 transcription factor, results in hypertrophic growth of isolated cardiac myocytes, which can be blocked by a signal-resistant HDAC4 mutant [88]. Moreover, studies using mice with cardiac overexpression of CaMKII (CaMKIIδ_C TG) show that HDAC4 is phosphorylated and exported out of the nucleus in response to CaMKII [86]. This raises the possibility that CaMKII-mediated phosphorylation of HDAC could be a downstream target of β-AR signaling. However, our own data using CaMKIIδ KO mice [89] and experiments by others using a novel HDAC inhibitor [90] provided evidence against a role for CaMKII or HDAC in β-AR induced hypertrophy. More studies are needed to determine whether HDACs are elements of the β-AR/CaMKII signaling cascade.

4. Role of CaMKII in β-AR signaling

Acute β-adrenergic activation causes the “fight-or-flight” response that makes the heart beat faster and stronger. Chronic β-adrenergic activation as observed in heart failure results in desensitization of β-adrenergic signaling with a decreased fight-or-flight response (i.e., exercise intolerance) and detrimental changes including hypertrophy, apoptosis and arrhythmias.

4.1. Acute effects of CaMKII on heart rate

The increase in heart rate following β-adrenergic stimulation results from enhancement of the discharge rate of sinoatrial node cells (SANC). The exact mechanism underlying chronotropic effects of β-adrenergic stimulation are only incompletely understood, but potential mechanisms involve stimulation of PKA and phosphorylation of target proteins including the LTCC, RyR, and the hyperpolarization-activated cyclic nucleotide-gated (HCN) channel gene family underlying the pacemaker or “funny” current (I_f). HCN4 is the most highly expressed isoform of the pore-forming subunits, and β-AR/cAMP directly activates HCN4 [91]. The role of I_f as a mediator of chronotropic effects of β-AR stimulation has been challenged by experiments showing that pharmacological I_f blockade [92] or deletion of HCN4 [93] failed to prevent sinus rate acceleration by isoproterenol. In contrast, CaMKII inhibition has been shown to lower the rate of SANC [94] and to block chronotropic responses of β-AR stimulation [95]. CaMKII inhibition is thought to prevent the effects of β-adrenergic stimulation on Ca^{2+} uptake and release from intracellular Ca^{2+} SR stores that are necessary for increasing diastolic depolarization rate in SANC [95]. This proposed mechanism is supported by studies showing that inhibition of the RyR using ryanodine prevents β-adrenergic augmentation of the diastolic depolarization rate [96], even if β-adrenergic effects on I_{Ca} current are preserved [97]. However, in another study ryanodine inhibited basal SANC firing rate but failed to block the β-adrenergic rate increase [98]. More information on the β-adrenergic control of cardiac pacemaker function can be found in a recent point/counterpoint discussion and a review article in earlier issues of this journal [99,100].

4.2. Acute effects of CaMKII on Ca^{2+} handling and contractility

As discussed above, CaMKII shares some of the major targets involved in Ca^{2+} regulation with PKA. These targets (LTCC, RyR, and PLN) are important regulators of cardiac excitation-contraction coupling. One would expect that both PKA and CaMKII could modulate contractility in response to β-AR stimulation, but most experimental evidence disproves a role of CaMKII in acute β-adrenergic modulation of contractility [13,16]. The Kranias group showed in PLN-deficient hearts and in transgenic mice expressing T17A or S16A mutant PLN on a PLN null background that phosphorylation of the PKA site Ser16 but not the CaMKII site Thr17 contributes to contractile responses to β-adrenergic agonists [101–103]. This observation is supported by kinetic experiments comparing phosphorylation of PKA and CaMKII target sites on PLN. These studies show a correlation of acute effects of isoproterenol on contractility with cAMP elevation and phosphorylation of the PKA target Ser16, whereas no correlation is found with the phosphorylation of the CaMKII site Thr17 [56].

Although it is generally accepted that PKA targets RyR Ser2808 to allow a greater calcium efflux from the SR [50,104,105], recent studies using a genetically modified mouse with a non-phosphorylatable RyR Ser2808 showed that phosphorylation of Ser2808 does not have a similar role in RyR2 channel function [106,107] or regulation of normal cardiac function by catecholamines [108,109]. It is known that endogenous CaMKIIδ associates directly with RyR2 and modulates RyR2 activity [110] and that phosphorylation of RyR2 by CaMKII increases channel activity and Ca^{2+} release [52,111,112]. Remarkably, CaMKII-dependent phosphorylation of RyR2 Ser2815 was demonstrated to be responsible for the β-AR induced increase in the channel activity [10], although no data are currently available that show effects of RyR Ser2815 phosphorylation on β-adrenergic regulation of cardiac contractility.

As mentioned above, RyR phosphorylation by CaMKII in the normal heart has as yet no known functional consequences in the response to β-adrenergic stimulation. However, pathophysiological conditions seem to change inotropic regulation. In the study by Wang et al. discussed earlier, chronic exposure to β-AR stimulation was demonstrated to cause a shift from PKA to CaMKII dependence of myocyte contractility [13]. Sustained inotropic responses of isolated myocytes to norepinephrine were largely PKA-independent but sensitive to specific CaMKII inhibitors or adenoviral expression of a dominant-negative CaMKII mutant [13]. Another study [113] found increased activity of CaMKII in mice with genetic disruption of the guanylyl cyclase-A (GC-A) receptor for atrial natriuretic peptide (ANP). In this model, responsiveness to β-AR stimulation was significantly increased and inhibition of CaMKII but not of PKA totally abolished the increased effects of β-adrenergic stimulation on cardiac contractility and Ca^{2+}-handling [113].

It has been suggested that CaMKII-dependent PLN Thr17 phosphorylation (like PKA mediated Ser16 phosphorylation) relieves inhibition of SERCA and subsequently more Ca^{2+} is pumped into the SR. This is supported by the finding that transgenic mice expressing a CaMKII inhibitory peptide targeted to the SR (AIP_4-LSR TG) display reduced PLN Thr17 phosphorylation and decreased SR Ca^{2+} uptake [60]. Indeed, the decay of Ca^{2+} transients was also found to be significantly slower in cardiomyocytes from mice expressing another CaMKII inhibitory peptide (AC3-I TG) [114]. The effect of AC3-I was abolished in the absence of PLN (AC3-I × $PLN^{-/-}$) [114]. AC3-I myocytes have a reduced SR Ca^{2+} content but a preserved contractile responses to β-AR stimulation [16], whereas AIP_4-LSR TG myocytes show reduced contractility in response to β-AR stimulation without changes in SR Ca^{2+} content [61]. Importantly, the latter study found a significant reduction in PKA target phosphorylation at RyR2809 and PLN Ser16 [61]. Taken together, the studies are in agreement with the observations by the Kranias group that contractile responses to β-AR stimulation do not require CaMKII-dependent phosphorylation of PLN [101–103].

There is an additional and potentially overriding effect of CaMKII on SR Ca^{2+} content mediated via its effect on RyR and its profound consequences on diastolic SR Ca^{2+} leak. Thus, mice with transgenic overexpression of cytoplasmic CaMKII show a *reduced* SR Ca^{2+} content [86,112] which can be explained by an increased SR Ca^{2+} leak through the RyR. Moreover, increases in the SR Ca^{2+} leak seen with chronic β-adrenergic stimulation or heart failure appear to be due to CaMKII activation as they are clearly rescued by inhibition of CaMKII but not by inhibition of PKA [12,43]. PLN phosphorylation does not seem to modulate the SR Ca^{2+} leak, because Ca^{2+} spark increases by CaMKII can be observed in both WT and PLN-KO myocytes [52,115], consistent with a direct effect of CaMKII on RyR.

Thus, it is not entirely clear if acute CaMKII activity reduces (via RyR) or increases (via PLN) SR Ca^{2+} content or if acute and chronic β-AR stimulation have different consequences on CaMKII-mediated Ca^{2+} handling.

4.3. Heart failure

Overexpression of the δ_C isoform of CaMKII causes heart failure in mice [53,112]. Heart failure is characterized by activation of the sympathetic nervous system, and subsequent stimulation of cardiac β-ARs. CaMKII expression and activity is also increased in human heart failure [116,117] and animal models of heart failure [53]. It is therefore possible that some of the detrimental effects of chronic β_1-AR stimulation are the result of CaMKII activation. Enhanced β_1-AR signaling leads to Ca^{2+} handling disturbances and cell death [118]. There is also evidence that activation of CaMKIIδ triggers cardiomyocyte apoptosis via the primary mitochondrial death pathway [2,17,119]. That the effect of β_1-AR stimulation on cell survival may be in part via CaMKII is suggested by evidence that inhibition of CaMKII protects against apoptosis during excessive β_1-AR stimulation [2,16,120], and that CaMKII inhibition has considerable impact on effects of chronic catecholamine stimulation leading to progression of structural heart disease [2,16,120,121]. These studies suggested that the proapoptotic actions of CaMKII were related to its ability to increase SR Ca^{2+} content because the benefits of CaMKII inhibition were not seen in PLN KO background [114,120]. Further information on the important role of PLN for the apoptotic actions of CaMKII is detailed in a recent review from Couchonnal and Anderson [122]. In addition, enhanced activity of CaMKII causes hyperphosphorylation of the RyR at Ser2815, which leads to an increased diastolic SR Ca^{2+} leak [112,123]. The markedly increased diastolic Ca^{2+} release from the SR could result in increased Ca^{2+} uptake by mitochondria, as the Ca^{2+} leak and mitochondrial Ca^{2+} filling are further enhanced when the SR Ca^{2+} store is repleted (Zhang et al., Circ Res, in revision). A recent screening study using 40 different agents (“bioprobes”) that induce apoptosis in tumor cells suggests that sustained activation of CaMKII has a common role in apoptosis not only in different mammalian cell types but also in response to different apoptotic stimuli [124].

Stimulation of cardiac β-ARs increases oxidative stress, which may have a role in catecholamine-induced heart disease [29–31,125]. ROS-dependent apoptosis [31] and extracellular matrix biosynthesis [29] following β-AR stimulation has been described. Notably, recently published work demonstrated that autonomous CaMKII activity is enhanced by pro-oxidant conditions [28]. However, CaMKII activation and apoptosis that is observed under chronic β-AR stimulation *in vivo* seems to occur independently of oxidative stress but rather depend on CaMKII-dependent increases of intracellular Ca^{2+} as described above [2,28]. Other events that are known to result from chronic CaMKII activation such as after-depolarizations as triggers of lethal ventricular arrhythmias [126] do, however, appear to be mediated by ROS.

Isoproterenol stimulation was recently demonstrated to induce significantly more arrhythmias in myocytes isolated from CaMKIIδ_C TG mice, a model in which SR Ca^{2+} leak is markedly increased [127]. Inhibition of CaMKII has also been shown to suppress isoproterenol-induced arrhythmias *in vivo* [45,127]. Further analysis demonstrated that the opening probability of L-

type Ca^{2+} channels, which is known to activate early afterdepolarizations (EADs), was significantly higher in myocytes from mice overexpressing a constitutively active form of CaMKIV vs. WT mice [45]. EADs can trigger arrhythmias and a CaMKII inhibitory peptide has been demonstrated to eliminate EADs [45]. Taken together, the data suggest that L-type Ca^{2+} channels and SR Ca^{2+} leak are major contributors to CaMKII-mediated arrhythmias and that this could contribute to the arrythmogenic effect of chronic sympathetic activation.

5. Conclusion

CaMKII is a multifunctional signaling molecule that is abundant in the heart and activated by β-AR stimulation. CaMKII targets cytoplasmic proteins important for Ca^{2+} handling such as PLN and the RyR but also transduces Ca^{2+} signals into the nucleus to regulate gene transcription. Chronic inhibition or gene deletion of CaMKII appear to have little effect on basal function of the heart or on acute responses to β-adrenergic stimulation but have a significant beneficial impact on cardiac function under pathological stresses known to be associated with chronic sympathetic activation. Thus, CaMKII has become a promising potential target for heart failure therapy.

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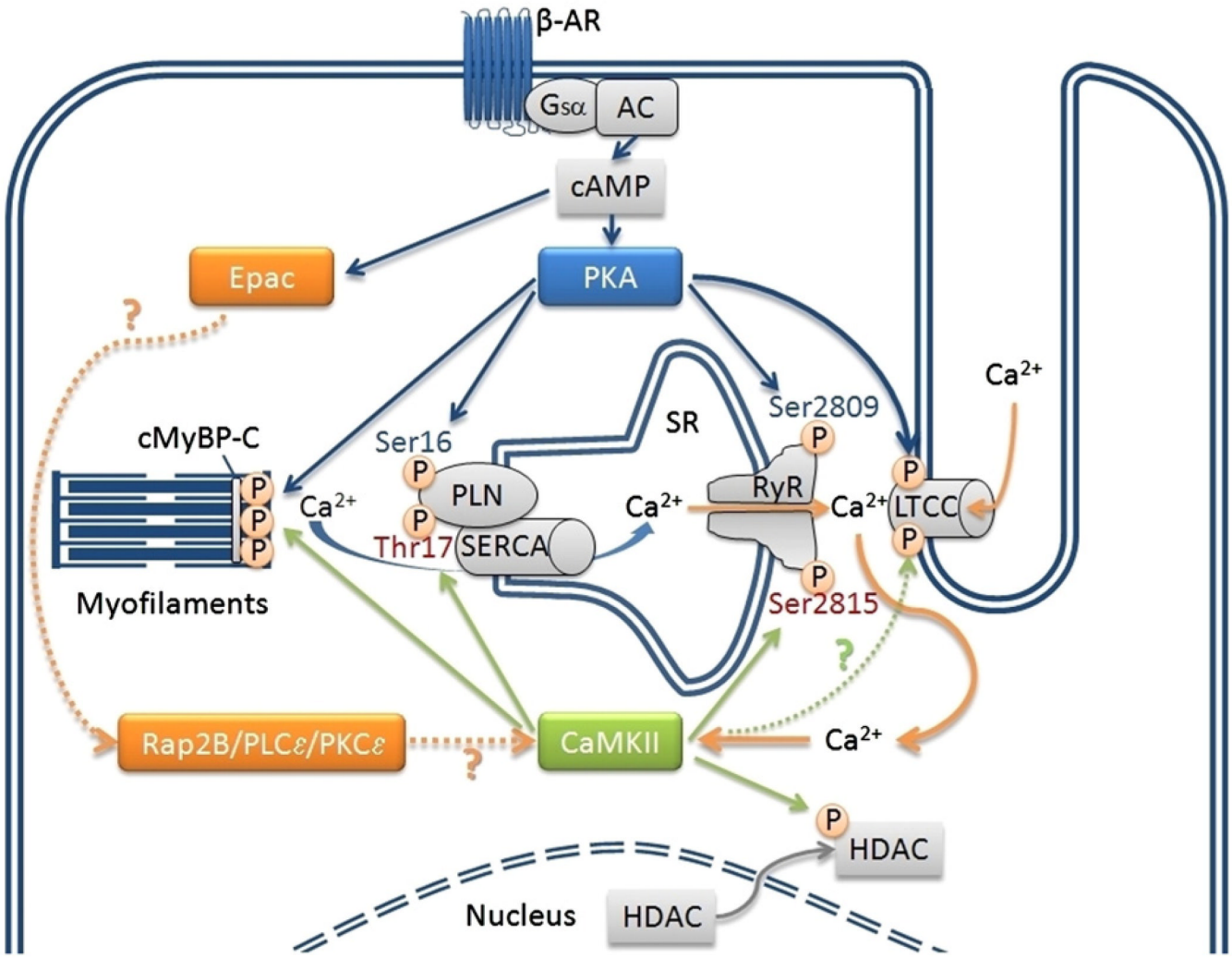

Fig. 1.

Activation of PKA and CaMKII by β-adrenergic receptor stimulation and phosphorylation of their targets. Question marks and broken lines indicate controversial pathways. Abbreviations: β-AR, β-adrenergic receptor; LTCC, L-type Ca^{2+} channel; Gsα, α subunit of the Gs-protein; AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; Epac, exchange protein activated by cAMP; SR, sarcoplasmic reticulum; PLN, phospholamban; SERCA, SR Ca^{2+}-ATPase; RyR, ryanodine receptor; CaMKII, Ca^{2+} and calmodulin-dependent kinase II; HDAC, histone deacetylase; cMyBP-C, cardiac myosin binding protein C; Rap2B, a RAS-related GTP-binding protein; PLCε, phospholipase Cε; PKCε, protein kinase Cε.