Physiological and unappreciated roles of CaMKII in the heart

Jan Beckendorf¹,²,³ · Maarten M. G. van den Hoogenhof¹,³ · Johannes Backs¹,³

Received: 28 March 2018 / Accepted: 11 June 2018 / Published online: 15 June 2018 © The Author(s) 2018

Abstract

In the cardiomyocyte, CaMKII has been identified as a nodal influencer of excitation–contraction and also excitation–transcription coupling. Its activity can be regulated in response to changes in intracellular calcium content as well as after several post-translational modifications. Some of the effects mediated by CaMKII may be considered adaptive, while effects of sustained CaMKII activity may turn into the opposite and are detrimental to cardiac integrity and function. As such, CaMKII has long been noted as a promising target for pharmacological inhibition, but the ubiquitous nature of CaMKII has made it difficult to target CaMKII specifically where it is detrimental. In this review, we provide a brief overview of the physiological and pathophysiological properties of CaMKII signaling, but we focus on the physiological and adaptive functions of CaMKII. Furthermore, special consideration is given to the emerging role of CaMKII as a mediator of inflammatory processes in the heart.

Keywords Calcium · Calmodulin · CaMKII · Cardiomyocyte · Inflammation · Apoptosis

Introduction

Heart failure is one of the most prevalent diagnoses upon hospital admission and, despite all therapeutic progress over the last decade, is still associated with a high rate of morbidity and mortality [54, 77]. In the diseased myocardium, CaMKII plays central roles in processes such as maladaptive remodeling [1, 3, 44, 45, 48, 50, 72, 115, 117], arrhythmogenesis [63], interstitial fibrosis [3, 45] and apoptosis [21, 22, 103, 112]. As such, CaMKII is a promising target for pharmacological inhibition and the development of inhibitory compounds is racing ahead [73]. Two compensatory mechanisms during heart failure are (a) an excessive production of catecholamines and (b) the activation of the renin–angiotensin–aldosterone system. For each of these mechanisms, CaMKII has been shown to play an integral role in conveying the following (mal)adaptive processes, leading to cardiac remodeling and heart failure [18, 30, 113]. However, while there is vast knowledge of the role of CaMKII in cardiac disease, the role of CaMKII in physiological processes is less well studied. This review aims at highlighting the sparse insights into the physiological role of CaMKII signaling in the heart and also its role in some underappreciated inflammatory processes in the heart.

CaMKII structure and activity

Calcium(Ca²⁺)/calmodulin(CaM)-dependent kinases (CaMK) are serine/threonine (Ser/Thr)-specific phosphokinases. They respond to changes in intracellular [Ca²⁺], which is the major second messenger inside the cardiomyocyte and indispensable for the coupling of membrane excitation with myofibril contraction, also termed excitation–contraction coupling (ECC) [55]. As free calcium ions are quickly removed from the cytosol during diastole, they can be bound by the Ca²⁺-sensor calmodulin [14] to allow the exertion of functions that last longer than just one depolarization, resp. one systole, especially on gene transcription or epigenetic regulation, often termed
excitation–transcription coupling (ETC) [8]. An increase of \([\text{Ca}^{2+}]\) inside the cardiomyocyte leads to the activation (and potentially overactivation) of calcium-dependent signaling. As a result, overall CaMKII activity is upregulated ~3-fold in human heart failure [42], and the expression rate of CaMKIIδ was shown to be increased ~2-fold [33].

The structure of the functional CaMKII enzyme is dodecameric, taking the form of two stacked hexameric rings [13]. Each monomer consists of an N-terminal catalytic domain and a C-terminal association domain. In between, an autoregulatory domain, which also includes the \(\text{Ca}^{2+}/\text{CaM}\) binding site, regulates the activation status through \(\text{Ca}^{2+}/\text{CaM}\)-binding and also by autophosphorylation [35]. \(\text{Ca}^{2+}/\text{CaM}\)-dependent activation of CaMKII is dependent on total \([\text{Ca}^{2+}]\), in a dose-dependent manner, but also on \(\text{Ca}^{2+}\) spark frequency, amplitude and duration, as well as the previous activation state [15]. When inactive, the catalytic domain is sterically blocked by the regulatory domain, a mode also referred to as the autoinhibitory state. CaMKII is activated upon \(\text{Ca}^{2+}/\text{CaM}\) binding to the CaM-binding site of the regulatory domain, leading to a conformational change, which exposes the kinase substrate and adenosine triphosphate (ATP) binding sites of the catalytic domain [81]. When one monomer enters the active state, the regulatory domains of neighboring CaMKII monomers become available for autophosphorylation at Thr287 (in CaMKIIδ, the exact numbering changes slightly between different CaMKII isoforms), furthering CaMKII activation and also blocking re-association of the catalytic domain with the autoinhibitory domain [35, 47], maintaining kinase activity even after dissociation of the \(\text{Ca}^{2+}/\text{CaM}\) complex. Autophosphorylation of Thr287 leads to another interesting effect called CaM trapping, in which CaM binding affinity is increased 1000-fold, keeping the \(\text{Ca}^{2+}/\text{CaM}\) complex in place and thus sustaining CaMKII activity under conditions of low \([\text{Ca}^{2+}]\) [61]. Further research unveiled other mechanisms of CaMKII activation via post-translational modifications (PTM) of the regulatory domain that are \(\text{Ca}^{2+}/\text{CaM}\) independent, such as oxidation of the Met281/282 residues by reactive oxygen species (ROS) [18] and S-Nitrosylation of Cys290 through a nitric oxide (NO)-dependent pathway [19], and O-GlcNAcylation at Ser279 during hyperglycemia [20]. However, these mechanisms still need the initial activation of CaMKII via the canonical \(\text{Ca}^{2+}/\text{CaM}\) binding. Eventually, CaMKII can be inactivated via dephosphorylation of Thr287 by protein phosphatase 2A (PP2A) or protein phosphatase 1 (PP1) [96]. Another phosphatase-independent mechanism for negative regulation of CaMKII activity exists via autophosphorylation of Thr305/306, preventing CaM from binding to the regulatory domain again once it dissociated from its binding site (CaM-capping) [78].

**CaMKII genes and splice variants**

The group of calcium/calmodulin-dependent kinases consists of three classes: CaMKI, CaMKII and CaMKIV. CaMKII is further distinguished by its four isoforms α, β, γ, δ—each isoform being encoded by a separate gene [98]. The expression rates of CaMKII isoforms differ between tissue types. CaMKIIα and β are predominantly expressed in neuronal tissue, while the CaMKIIδ and γ isoforms can be found in cells of almost any differentiation [34]. CaMKIIδ and γ are the main isoforms found in cardiac tissue, with the δ isoform outweighing the γ isoform ~2.5-fold [3].

All CaMKII genes are subjected to alternative splicing, but CaMKIIδ splicing is most well studied in the heart. Alternative splicing of CaMKIIδ results in at least 11 different splice variants, among which the δA, δB, δC and δ9 are most seen in the heart (Fig. 1) [24]. The δA splice variant preferentially localizes to t-tubules, sarcolemmal and nuclear membranes, and is implicated in the formation of maladaptive cardiac hypertrophy after catecholaminergic stimulation [48, 111]. CaMKIIδA uniquely contains a nuclear localization sequence (NLS) and thus predominantly localizes to the nucleus, while CaMKIIδC mainly localizes to the cytosol [95]. At the moment, very little is known about CaMKIIδγ, but as it resembles CaMKIIδA the most, it may function in a similar manner. Three splice variants of CaMKIIγ have been found in the heart [88], but in contrast to the CaMKIIδ splice variants, the respective function of each CaMKIIγ splice variant in the heart is unknown. Each completely assembled dodecamer is constructed of different isoforms and splice variants. It is thought that the relative predominance of a certain splice variant in the heteromultimer might confer the target specificity for the respective cell compartment of the entire enzyme [62]. There is evidence that the differential compartmentalization of the splice variants also reflects differences in function, as the δB variant may predominantly regulate transcription and the δC variant may rather influence excitation–contraction coupling [114]. The different functions and relative importance of the splice isoforms of CaMKIIδ are, however, far from clear. For instance, it has been shown that δC is also able to block the nuclear import of histone deacetylase 4 (HDAC4), thereby possibly affecting gene expression as efficient as the δB variant [4, 116]. Systematic analyses of these different splice variants in different stages of cardiac development and disease are therefore awaited with great interest.

CaMKIIδ splicing is regulated by at least two different splicing factors, ASF/SF2 and Rbm20 [26, 111]. Members of the Rhox protein family and SC35 have also been implicated in CaMKIIδ splicing, but their in vivo
relevance is less clear [29]. Interestingly, during development CaMKIIδ switches from the CaMKIIδA splice variant, to the CaMKIIδB and CaMKIIδC variants, and loss of either ASF/SF2 or Rbm20 leads to persistent expression of fetal CaMKIIδA. It has been hypothesized that CaMKIIδA is necessary for enhanced L-type calcium current in neonatal cardiomyocytes, as they rely on L-type calcium current instead of calcium-induced calcium release (CICR) for contraction [27, 111]. While it is not yet known how CaMKIIδA enhances L-type calcium current, this hypothesis is in line with the observed increased calcium transients in ASF/SF2 knockout (KO) mice and CaMKIIδA-TG mice [111]. Interestingly, this effect seems to be gender dependent, as only male ASF/SF2 KO and CaMKIIδA-TG mice were affected. Very recently, van den Hoogenhof et al. found that Rbm20 KO mice have an intracellular Ca²⁺ overload, which leads to spontaneous Ca²⁺ releases from the SR [101]. It seems likely that this underlies the increased risk of arrhythmias in RBM20 mutation carriers. Interestingly, this Ca²⁺ overload was due to increased L-type Ca²⁺ current density, and as loss of Rbm20 also induces a shift to the fetal CaMKIIδA isoform, this is completely in line with the hypothesized function of CaMKIIδA.

CaMKIIδB is involved in remodeling via the epigenetic regulator HDAC4 during pathological pressure overload [4, 116]. However, it was also suggested that CaMKIIδB might mediate cardioprotective effects, as it strongly suppresses cardiomyocyte apoptosis after doxorubicin treatment and during oxidative stress [51] [74]. Nevertheless, CaMKIIδB transgenic mice develop hypertrophy and moderate cardiac dysfunction at 4 months of age [115]. Transgenic overexpression of CaMKIIδC in mice, on the other hand, results in a rapid progression of heart failure and premature death [117], and Sossalla et al. demonstrated the role of CaMKIIδC in diastolic dysfunction and arrhythmogenesis [93]. However, in contrast to these previous findings, the collaborative work of our laboratory with Wolfgang Linke pointed to a reduction in passive stiffness of cardiomyocytes after phosphorylation of the sarcomeric structure protein titin by CaMKIIδC, improving diastolic filling, an effect that may be partially beneficial in diastolic dysfunction [28]. The latter finding warrants further investigations to explore its functional relevance in in vivo situations including diastolic dysfunction. However, functional redundancy among the different CaMKII genes and perhaps with other related kinases including protein kinase D complicate such studies because they require breeding of different mouse models.

**Physiological and adaptive functions of CaMKII**

As CaMKII is a ubiquitously expressed and multifunctional kinase, its function and importance have been studied in a multitude of tissues. Outside the heart, CaMKII is has been shown to be critically involved in vital processes like memory formation through long-term potentiation [2], hepatic glucose production and insulin signaling [69, 70], vascular smooth muscle cell function [99], cell cycle progression and fertility [5, 39], as well as the immune system [10]. In the heart, the role of CaMKII under conditions of pathological cardiac stress has been studied extensively. However, relatively little is known about the role of CaMKII in the non-diseased heart after physiological stimuli, as well as its possible adaptive roles in the diseased heart. The newly
generated conditional KO models [3, 5] of the two ubiquitously expressed CaMKII genes δ and γ might provide a toolbox that allows to identify unknown essential CaMKII functions.

CaMKII is recognized as an instrument of the cell for the fine-tuning of its intracellular calcium content, especially concerning the ECC in myocytes. During the plateau phase of the action potential, calcium shifts into the cell through L-type calcium channels (LTCC), which leads to a relatively low increase of subsarcolemmal calcium in the dyadic cleft between the sarcolemma of the T-tubes and the sarcoplasmic reticulum. There, each LTCC is juxtaposed by a cluster of ryanodine receptors (RyR2). The initial calcium influx is followed by an amplifying mechanism called calcium-induced calcium release (CICR), during which even more calcium is quickly released from the sarcoplasmic reticulum through the ryanodine receptor, boosting [Ca<sup>2+</sup>].

Thereby, the binding of free cytosolic calcium with troponin C is made possible, which then leads to the conformational change of the tropomyosin/actin complex and enables myosin binding, ultimately leading to myofilament contraction [92]. During diastole, free calcium is rapidly removed from the cytosol, either by transport into the extracellular space through the sodium/calcium exchanger (NCX) or by reuptake into the SR via the SR-Ca<sup>2+</sup>-ATPase (SERCA).

These processes can be regulated by CaMKII: CaMKII can, for example, phosphorylate several subunits of the LTCC, thereby increasing Ca<sup>2+</sup>-dependent facilitation of the LTCC [36, 43]. In addition, CaMKII phosphorylates the sarcoplasmic reticulum (SR) membrane protein-complex phospholamban (PLN) at Thr17 [87], leading to increased calcium reuptake from the cytosol into the SR via SERCA2a [59]. Lastly, the ryanodin receptor 2 (RyR2), which is located in the sarcoplasmic reticulum membrane, is phosphorylated by CaMKII at Ser2809 [107] and more importantly Ser2814 [102, 104], leading to reduced SR Ca<sup>2+</sup> leak into the cytosol. The details of CaMKII and its role in ECC and ETC, however, are beyond the scope of this review and both have previously been reviewed in depth by many investigators including Lars Maier [55] and Donald Bers [8], respectively.

CaMKII is not only pivotal for calcium handling in ECC and ETC, but is also required for the increase in heart rate (HR) after β-adrenergic stimulation, also known as the flight or flight response [109]. Sinoatrial node (SAN) cells rely on an inward ‘pacemaker’ current through HCN4, leading to faster action potential generation, but HCN4 KO mice retain their ability to increase HR after β-adrenergic stimulation. Wu and colleagues showed that activation of CaMKII in SAN cells enhances SR Ca<sup>2+</sup> filling and release, and increases the diastolic depolarization rate. This leads to faster action potential generation, independent of HCN4 current. Interestingly, CaMKII inhibition only affects HR after β-adrenergic stimulation, and not at baseline. It must be noted that this effect did not depend on a single CaMKII in PLB or RyR2, but rather that the concerted action on multiple phosphorylation targets decreases SR Ca<sup>2+</sup> content below a certain threshold which seems to be required for the flight or flight response [110].

A recent study showed that CaMKII is centrally involved in the adaptive contractile response after aerobic training, and therefore indispensable for the adequate response of the heart to a physiological stimulus [12]. Mechanistically, this effect was shown to depend on increasing levels of insulin-like growth factor 1 (IGF-1) after exercise, which leads to activation of the nitric oxide (NO) synthase 1 (NOS-1) through the PI3K/Akt pathway. This, in turn, leads to activation of CaMKII, putatively through the NO-dependent S-nitrosylation of Cys290, resulting in the enhancement of calcium cycling through SERCA2a and the desirable effects of increased inotropy and lusitropy. Interestingly, blockade of CaMKII with the inhibitory peptide AC3-I abolished the effects on contractility and relaxation, but not the cardiomyocyte hypertrophy.

Along those lines, our laboratory, using CaMKIIγ/CaMKIIδ double knockout (DKO) mice, showed that pathological and physiological cardiac hypertrophy in mice was not primarily CaMKII dependent, but rather attributable to the calcineurin (CnA)–NFAT axis, while CaMKII was responsible for maladaptive effects, i.e., systolic and diastolic dysfunction [44]. A similar observation that hypertrophy was independent of CaMKII while maladaptive remodeling did require CaMKII was made by the group of Joan Heller Brown [50]. At baseline, CaMKIIγ/CaMKIIδ DKO mice exhibit a slight increase in contractile force, but even though PLN-Thr17 and RyR2-Ser2814 were markedly hypophosphorylated, no changes in cellular Ca<sup>2+</sup> handling could be detected [44]. While this suggests that CaMKII is dispensable for normal cardiac function, CaMKII is also involved in the adaptive response after physiological stress. Upon exercise, CaMKII expression in control mice was unaltered, but activity was decreased by 30%. Even though control mice had a hypertrophic response, as indicated by increased heart weight/body weight (HW/BW) ratios and cardiomyocyte hypertrophy, this did not affect cardiac function. In CaMKIIγ/CaMKIIδ DKO mice this response was exaggerated, and the CnA target gene RCAN1–4 was excessively upregulated, but cardiac function was also not affected. However, decreased CaMKII activity decreases phosphorylation of the autoinhibitory Ser411 phosphorylation site of CnA, suggesting that CaMKII is necessary to inhibit overactivation of calcineurin.

Conversely, Ole Kemi and co-workers previously showed increased cardiac contractility and Ca<sup>2+</sup> cycling after aerobic interval training in adult mice, and inhibition of CaMKII using the autacamtide-2 related inhibitory peptide II
(AIP) abolished these effects [41]. These animals also did not show an increase of overall CaMKII expression, but in this case CaMKII activity, as assessed by P-Thr287-CaMKII and P-Thr17-PLN, was increased. In human skeletal muscle, P-Thr287-CaMKII is increased as early as 5 min after the start of the exercise, and activity is increased after 40 min [80]. Endurance training of human skeletal muscle also increases P-Thr287-CaMKII and activity, but here P-Thr17-PLN was unaltered [79]. Currently, there is no satisfactory answer to these seemingly contradictory results, but in these studies CaMKII activity has been measured in different and indirect assays, and exercise regimens were different, which could explain the discrepancies.

Another beneficial function of CaMKII is that recovery from acidosis depends on acute CaMKII activation. Acidosis, the lowering of pH, which can be of clinical significance during myocardial infarction and cardiac ischemia, decreases contractile performance and alters intracellular calcium handling [68]. On the electrophysiological level, acidosis increases extrusion of H+ from the cardiomyocyte by the Na+/H+ exchanger, which increases intracellular [Na⁺]. This, in turn, increases diastolic [Ca²⁺], through the reverse mode of NCX. In cardiomyocytes, this activates CaMKII, which can then phosphorylate PLN to increase Ca²⁺ re-uptake by SERCA2a, ultimately leading to increased SR Ca²⁺ content and increased Ca²⁺ transients [60]. The increase in Ca²⁺ transients is pivotal in overcoming the decreased contractility during acidosis, and CaMKII activation has proven to be necessary for this coping mechanism, both in vitro and in vivo [65, 68]. However, acute activation of CaMKII also has adverse effects; for example, ethanol and doxorubicin can acutely activate CaMKII, which ultimately leads to an increased SR Ca²⁺ leak that seems to be pro-arrhythmic [64, 82]. Ethanol and doxorubicin both increase ROS production, which consequently can activate CaMKII by oxidation. Activated CaMKII is known to promote diastolic SR Ca²⁺ leak, for example by hyperphosphorylation of RyR2, which increases the open probability of the channel. Ultimately, this can repress Ca²⁺ transients and contractility and serve as a basis for arrhythogenic effects. It must be noted that CaMKII and protein kinase A (PKA) share a number of phosphorylation targets, among which are RyR2 and PLN [23, 110]. RyR2, for example, can be phosphorylated by CaMKII at Ser2815 and by PKA at Ser2809, and both phosphorylation events increase the open probability of the RyR2 channel and are therefore pro-arrhythmic. Fisher et al. have recently shown that during hypertrophy, both CaMKII- and PKA-dependent phosphorylations of RyR2 are increased, which may induce SR Ca²⁺ leak, but during the transition from hypertrophy to heart failure, only CaMKII-dependent phosphorylation of RyR2 is increased [23]. Discussing the differential roles of CaMKII vs. PKA in the regulation of their phosphorylation targets is beyond the scope of this review, but extensive literature on this subject exists (see for example Johnston et al. [37] or Marx et al. [57]).

Nevertheless, the beneficial sides of short-term or acute activation of CaMKII need not be disregarded, and further studies are needed to unravel the relative contributions of CaMKII in the different phases of the adaptive response of heart and skeletal muscle to physiological stress. It will be interesting to identify and investigate the targets of CaMKII at different time points after physiological stimuli, to discern what mechanisms, be it calcium cycling remodeling, gene regulation, or metabolic remodeling, are most prominently affected.

### The role of CaMKII in apoptosis and necroptosis

While apoptosis (or programmed cell death) is an important physiological mechanism of well-ordered organ development, it is also one of the pathophysiological hallmarks of myocardial remodeling in heart failure where it entails detrimental effects on cardiac contractility through cell loss [40]. The role of CaMKII in apoptotic signaling in non-cardiac cancer cells was first published by Wright et al. [108] and, a few years later, Zhu et al. demonstrated that CaMKII was essential for cardiomyocyte apoptosis after beta-adrenergic overstimulation [119]. Since then, a huge body of work supports the pro-apoptotic properties of CaMKII signaling as recently reviewed by Feng and Anderson [22]. However, these experiments were mostly done using chemical or peptide-based kinase inhibition (AIP, KN-93, AC3-I), which are prone to several limitations (as discussed in [105]). In these studies, CaMKII inhibition seemed to be clearly anti-apoptotic. However, new studies indicated different roles of CaMKIIδ splice variants in apoptosis, when Peng et al. and Little et al. confirmed pro-apoptotic properties only for CaMKIIδC, but unexpectedly found anti-apoptotic properties for CaMKIIδB after oxidative and doxorubicin-induced myocardial damage [51, 74]. This seemingly clear-cut picture of good and evil became muddled when our group aimed to dissect the individual roles of CaMKIIδ, CaMKIIγ and Little et al. confirmed pro-apoptotic properties only for CaMKIIδC, but unexpectedly found anti-apoptotic properties for CaMKIIδB after oxidative and doxorubicin-induced myocardial damage [51, 74]. This seemingly clear-cut picture of good and evil became muddled when our group aimed to dissect the individual roles of CaMKIIδ, CaMKIIγ and CaMKIIβ isoform or splice variant. In contradiction, Ling et al. demonstrated a clear increase of apoptotic cell death after I/R, which was abrogated by CaMKIIδC knockout [50]. There-
circumstances, even the chaotic process of necrosis may underlie some cellular control. This regulated form of necrosis has therefore been termed necroptosis as a portmanteau of necrosis and apoptosis [46]. Necroptosis can be triggered by activation of receptor-interacting protein 3 (RIP3), a protein phosphokinas that has CaMKII as a substrate [118]. This is a unique finding, as CaMKII was previously not known to be phosphorylated by any other kinase than itself. Disruption of RIP3 or CaMKII signaling leads to a marked reduction of cell death after I/R or doxorubicin treatment. CaMKII was previously suggested to influence the opening of the mitochondrial permeability transition pore (mPTP) by increasing inner membrane mitochondrial calcium uniporter currents ($I_{\text{MCU}}$), leading to depolarization of the mitochondrial inner membrane and ultimately cell death [38]. It may be speculated that through its involvement in necroptosis, CaMKII may also play a regulative role, possibly by preventing uncontrolled necrosis during cardiac injury.

**CaMKII signaling in inflammation**

Recent works have placed CaMKII signaling in the middle of inflammatory processes. In immune cells, CaMKII plays a major role in the activation of T cells and the formation of T cell memory mirroring the function of CaMKII in memory formation in the brain [9, 10, 66]. Furthermore, CaMKII signaling in the immune system was found to be responsible for the pro-inflammatory cytokine production in macrophages [52, 75] and for dendritic cell function [32]. CaMKII activity is also associated with the propagation of asthmatic bronchitis through pro-inflammatory action in the airway epithelium, smooth muscle cells and mast cells and this was mostly ROS dependent [84, 86]. However, CaMKII can also be activated downstream of inflammatory stimuli such as toll-like receptor (TLR) activation [91] or interleukin-10 (IL-10) signaling [75].

In the heart, CaMKII signaling is intricately involved in the propagation of ischemic and reperfusion-associated damage to the heart muscle, thereby influencing the degree of inflammatory response and, thus, scar formation and cardiac function. The importance of CaMKII in these processes, however, has been under debate, and opposing results have been reported. Some work on this subject was done by the group of Joan Heller Brown, where in the wake of 60 min ischemia with following reperfusion for up to 24 h, cardiomyocyte-CaMKII was discovered to phosphorylate and thereby activate I kappa B kinase (IKK), leading to de-repression of nuclear factor kappa B (NF-κB), a central regulator of inflammation [49]. This effect could be diminished by inhibition of IKK, as well as genetic deletion of CaMKIIδ, leading to reduced infiltration of the ischemic muscle area by macrophages and eventually resulting in attenuated scar size and improved pump function. In a follow-up study, the respective roles of the splice variants δB and δC in the setting of injury/reperfusion (I/R) damage were examined [25]. Mice that overexpressed CaMKIIδC in a background of global CaMKIIδ deletion showed increased infarct size and systolic dysfunction. The opposite was observed in mice with isolated CaMKIIδB overexpression, where infarct size was even smaller than in the complete CaMKIIδ KO, an observation that strengthens the notion that CaMKIIδB can exert protective effects through suppression of cardiomyocyte apoptosis [51, 74]. Furthermore, it was shown that the activation of the CaMKIIδC–IKK–NF-κB axis leads to increased expression of tumor necrosis factor alpha (TNFα), and inhibition of either IKK or TNFα was sufficient to reduce infarct size [25]. This pathway was previously also implied in other models of cardiac disease [89, 90]. However, it must be noted that clinical trials, examining the potential of a blockade of the mentioned pathways in the setting of myocardial infarction or heart failure so far, were disappointing, both for NF-κB inactivation through the administration of glucosteroids [11] and after treatment with the TNFα blocker etanercept [56, 71].

However as mentioned above, in a similar I/R study from our group, Weinreuter and co-workers did not observe a difference in infarct size or apoptosis 1 day after I/R in CaMKIIδ KO, CaMKIIγ KO and CaMKIIγ/δ DKO mice, and also after re-expression of CaMKIIδ or CaMKIIδC. Only at 5 weeks after I/R, CaMKIIγ/δ DKO mice showed a reduced infarct size and improved cardiac function. This effect was associated with attenuated leukocyte infiltration and chemoattractant signaling in the hearts of CaMKIIγ/δ DKO mice, in particular in the time period from 1 to 5 days after I/R. Specifically, loss of CaMKII decreased the cardiomyocyte-intrinsic expression and secretion of the chemokines C–C motif ligand (CCL) 2 and 3, and thereby decreased scar area through diminished attraction of inflammatory cells (Fig. 2) [105]. The discrepancy between these studies may be due to the utilization of different KO strategies or the dissimilar genetic background of the animals, and future studies to investigate the potential reasons underlying the different results are needed. Since still little is known about CaMKII in the setting of chronic post-ischemic heart failure after the cessation of acute inflammatory processes, further research into the role of CaMKII in chronic post-ischemic heart failure is urgently warranted. The inflammatory processes that occur in the heart after MI have different stages, with different cell types involved, and the chemoattractant CCL2 is needed in the first stage to attract Ly-6Chigh monocytes [97]. Ly-6Chigh monocytes are required during the initial response, but can be detrimental if they persist too long [97]. Increased understanding of these processes, and how CaMKII is involved, might lead to new CaMKII-based therapeutic strategies that point to a specific treatment
period after ischemic injury which aims to avoid infiltration of specific subsets of leukocytes into the myocardium.

CaMKII may also play an ambiguous role in angiogenesis during inflammatory conditions. Westra et al. showed that inhibition of CaMKII leads to reduced expression of hypoxia-inducible factor 1α (HIF-1α) in macrophages, thereby also decreasing the expression of vascular-endothelial growth factor (VEGF) and possibly reducing angiogenesis [106]. Additional evidence was recently provided by Banumathi et al., who showed that retinal angiogenesis is critically dependent on CaMKII, and inhibition of CaMKII with KN-93 decreased retinal angiogenesis [6]. However, after myocardial infarction, increased angiogenesis is highly desirable [85] and a potential therapeutic CaMKII inhibition might be disadvantageous regarding revascularization and collateralization of hypoxic areas.

**CaMKII in infectious disease**

Of note, CaMKII signaling was discovered to be involved in the progression of Chagas’ disease by enabling heme-induced cell proliferation of the *Trypanosoma cruzi* epimastigotes [67, 94]. Chagas disease is a potentially deadly disease afflicting many Latin American regions and its incidence is currently rising due to increased population mobility and non-vectorial transmission [76, 83]. Very limited therapeutic options are available for the treatment of this disease, especially during its chronic phase [83]. Here, pharmacological inhibition of CaMKII might therefore serve as a potential anti-infective strategy. An interesting question arising from this observation is whether CaMKII signaling might also be involved in the propagation of Chagas-associated cardiomyopathy that develops in up to 30% of patients [100], considering that an effect of *T. cruzi* on cardiomyocyte calcium handling is already known [7]. This thought is especially tantalizing, as it was shown that the related *Trypanosoma brucei*, which may also confer myocardial disease, can directly induce CaMKII-mediated proarrhythogenic SR calcium leak in cardiomyocytes [17] and an upregulation of the chemokines CCL2 and CCL3 was found in *T. cruzi*-associated cardiomyopathy [53], which, we know now, is driven by CaMKII [105]. Combining Chagas disease with CaMKII conditional KO mouse models might answer this intriguing question in the future.

**Conclusions**

The role of CaMKII as a promoter of adverse cardiac remodeling, dysfunction, arrhythmia and inflammatory processes is relatively clear. However, its role in the cardiovascular physiology in response to benign stress, e.g., endurance training, is a more ambiguous one. In addition, some works even describe cardioprotective effects of CaMKII activation under certain pathological stimuli, and the essential roles of CaMKII outside the heart should not be ignored, as these poorly understood effects could have a huge impact on drug development programs and would favor a CaMKII target-specific approach over enzymatic CaMKII inhibition. Overall, the beneficial effects of acute or short-term activation should not be disregarded and, though the maladaptive effects of sustained CaMKII activation are well studied, future studies are needed to discern if CaMKII really is the foe it has been made out for or maybe has a more acute, but neglected friendly side.

**Funding** J.Ba. was supported by the SFB 1118 (Deutsche Forschungsgemeinschaft, DFG), the DZHK (Deutsches Zentrum für Herz-KreislauForschung—German Centre for Cardiovascular Research) and the BMBF (German Ministry of Education and Research).

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no competing interests.
Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

1. Anderson ME, Brown JH, Bers DM (2011) CaMKII in myocardial hypertrophy and heart failure. J Mol Cell Cardiol 51:468–473. https://doi.org/10.1016/j.yjmcc.2011.01.012

2. Ataeei N, Sabzghabaei AM, Movahedian A (2015) Calcium/calmodulin-dependent protein kinase II is a ubiquitous molecule in human long-term memory synaptic plasticity: a review. Int J Prev Med 6:88. https://doi.org/10.4103/2008-7802.16483

3. Backs J, Backs T, Neef S, Kreusser MM, Lehmann LH, Patrick DM, Grueter CE, Qi X, Richardson JA, Hill JA, Katus HA, Bassel-Duby R, Maier LS, Olson EN (2009) The delta isoform of CaMK kinase II is required for pathological cardiac hypertrophy and remodeling after pressure overload. Proc Natl Acad Sci USA 106:2342–2347. https://doi.org/10.1073/pnas.0813013106

4. Backs J, Song K, Bezprozvannaya S, Chang S, Olson EN (2006) CaMKII selectively signals to histone deacetylase 4 during cardiomyocyte hypertrophy. J Clin Investig 116:1853–1864. https://doi.org/10.1172/JIC27438

5. Banumathi E, O’Connor A, Gurunathan S, Sipson DA, McGeown JG, Curtis TM (2011) VEGF-induced retinal angiogenic signaling is critically dependent on Ca2(+)(+) signaling by Ca2(2)(+)calmodulin-dependent protein kinase II. Invest Ophthalmol Vis Sci 52:3103–3111. https://doi.org/10.1167/iovs.10-6574

6. Barr SC, Han W, Andrews NW, Lopez JW, Ball BA, Panna-becker TL, Gilmour RF Jr (1996) A factor from Trypanosoma cruzi induces repetitive cytosolic free Ca2(+) transients in isolated primary canine cardiac myocytes. Infect Immun 64:1770–1777

7. Bers DM (2011) Ca(2)(+)calmodulin-dependent protein kinase II regulation of cardiac excitation–transcription coupling. Heart Rhythm 8:1101–1104. https://doi.org/10.1542/hrrthm.2011.01030

8. Boubali S, Liopeta K, Virgilio L, Mavrothalassitis G, Dimitracopoulos G, Paliogianni F (2012) Calcium/calmodulin-dependent protein kinase II regulates IL-10 production by human T lymphocytes: a distinct target in the calcium dependent pathway. Mol Immunol 52:51–60. https://doi.org/10.1016/j.molimm.2012.04.008

9. Bui JD, Calbo S, Hayden-Martinez K, Kane LP, Gardner P, Hedrick SM (2000) A role for CaMKII in T cell memory. Cell 100:457–467

10. Bulkley BH, Roberts WC (1974) Steroid therapy during acute myocardial infarction. A cause of delayed healing and of ventricular aneurysm. Am J Med 56:24–250

11. Burgos JL, Yeves AM, Barrena JP, Portiansky EL, Vila-Petroff MG, Ennis IL (2017) Nitric oxide and CaMKII: critical steps in the cardiac contractile response To IGF-1 and swim training. J Mol Cell Cardiol 112:16–26. https://doi.org/10.1016/j.yjmcc.2017.08.014

12. Chao LH, Stratton MM, Lee IH, Rosenberg OS, Levitz J, Mandell DJ, Kortemme T, Groves JT, Schulman H, Kuriyan J (2011) A mechanism for tunable autoinhibition in the structure of a human Ca2+/calmodulin-dependent kinase II holoenzyme. Cell 146:732–745. https://doi.org/10.1016/j.cell.2011.07.038

13. Chin D, Means AR (2000) Calmodulin: a prototypical calcium sensor. Trends Cell Biol 10:322–328

14. De Koninck P, Schulman H (1998) Sensitivity of CaMK kinase II to the frequency of Ca2(+) oscillations. Science 279:227–230

15. De Koninck P, Schulman H (2000) A role for CaMKII in T cell memory. Cell 97:305–314

16. Eefting F, Rensing B, Wigman J, Pannekoek WJ, Liu WM, Cramer MJ, Lips DJ, Doevendans PA (2004) Role of apoptosis in reperfusion injury. Cardiovasc Res 61:414–426. https://doi.org/10.1016/j.cardiores.2003.12.023

17. Elliott EB, McCarroll D, Hasumi H, Welsh RM, Spitz DR, Schwarz MA, Colbran RJ, Mohler PJ, Anderson ME (2008) A dynamic pathway for calcium-independent activation of CaMKII by methionine oxidation. Cell 133:462–474. https://doi.org/10.1016/j.cell.2008.02.048

18. Erickson JR, Nichols CB, Uchinoumi H, Stein ML, Bossuyt J, Bers DM (2015) S-Nitrosylation induces both autonomous activation and inhibition of calcium/calmodulin-dependent protein kinase II delta. J Biol Chem 290:25646–25656. https://doi.org/10.1074/jbc.M115.650234

19. Erickson JR, Pereira L, Wang L, Han G, Ferguson A, Dao K, Copeland RJ, Despa F, Hart GW, Ripplinger CM, Bers DM (2013) Diabetic hyperglycaemia activates CaMKII and arrhythmias by O-linked glycosylation. Nature 502:372–376. https://doi.org/10.1038/nature12537

20. Federico M, Portiansky EL, Sommese L, Alvarado PJ, Blanco PG, Zanuzzi CN, Sedman J, Kaetzel M, Wehrs XHT, Mattiazi A, Palomeque J (2017) Calcium–calmodulin-dependent protein kinase mediates the intracellular signalling pathways of cardiac apoptosis in mice with impaired glucose tolerance. J Physiol 595:4089–4108. https://doi.org/10.1113/JP273714

21. Feng N, Anderson ME (2017) CaMKII is a nodal signal for multiple programmed cell death pathways in heart. J Mol Cell Cardiol 103:102–109. https://doi.org/10.1016/j.yjmcc.2016.12.007

22. Fischer TH, Herting J, Tirilomis T, Renner A, Neef S, Toische K, Ellenberger D, Forster A, Schmitto JD, Gummert J, Schon-dube FA, Hasenfuss G, Maier LS, Sossalla S (2013) Ca2(+)calmodulin-dependent protein kinase II and protein kinase A differentially regulate sarcoplasmic reticulum Ca2(+) leak in human cardiac pathology. Circulation 128:970–981. https://doi.org/10.1161/CIRCULATIONAHA.113.001746

23. Gray CB, Heller Brown J (2014) CaMKIIdelta subtypes: localization and function. Front Pharmacol 5:15. https://doi.org/10.3389/fphar.2014.00015

24. Gray CB, Suetomi T, Xiang S, Mishra S, Blackwood EA, Glem-botski CC, Miyamoto S, Westenbrink BD, Brown JH (2017) CaMKIIdelta subtypes differentially regulate infarct formation following ex vivo myocardial ischemia/reperfusion through NF-kappaB and TNF-alpha. J Mol Cell Cardiol 103:48–55. https://doi.org/10.1016/j.yjmcc.2017.01.002

25. Guo W, Schafer S, Greaser ML, Vakeel P, Klaassen S, Gerull B, Thierfelder L, Regitz-Zagrosek V, Hacker TA, Sauge KW, Dec GW, Ellinor PT, MacRae CA, Spallek B, Fischer R, Perrot A, Ozcelik C, Saar K, Hubner N.
Gotthardt M (2012) RBM20, a gene for hereditary cardiomyopathy, regulates titin splicing. Nat Med 18:766–773. https://doi.org/10.1038/nm.2693

27. Haddock PS, Coetzee WA, Cho E, Porter L, Katoh H, Bers DM, Jafri MS, Artman M (1999) Subcellular [Ca\(^{2+}\)]\(_i\) gradients during excitation–contraction coupling in newborn rabbit ventricular myocytes. Circ Res 85:415–427

28. Hamdani N, Kryskij J, Kreusser MM, Neef S, Dos Remedios CG, Maier LS, Kruger M, Backs J, Linke WA (2013) Crucial role for Ca\(^{2+}\)+/calmodulin-dependent protein kinase-II in regulating diastolic stress of normal and failing hearts via titin phosphorylation. Circ Res 112:664–674. https://doi.org/10.1161/CIRCRESAHA.111.300105

29. Han J, Ding JH, Byeon CW, Kim JH, Hertel KJ, Jeong S, Fu XD (2011) SR proteins induce alternative exon skipping through their activities on the flanking constitutive exons. Mol Cell Biol 31:793–802. https://doi.org/10.1128/MCB.01117-10

30. He BJ, Joiner ML, Singh MV, Luczak ED, Swaminathan PD, Han J, Ding JH, Byeon CW, Kim JH, Hertel KJ, Jeong S, Fu XD (2011) SR proteins induce alternative exon skipping through their activities on the flanking constitutive exons. Mol Cell Biol 31:793–802. https://doi.org/10.1128/MCB.01117-10

31. Heijmen J, Voigt N, Wehrens XH, Dobrev D (2014) Calcium dysregulation in atrial fibrillation: the role of CaMKII. Front Pharmacol 5:30. https://doi.org/10.3389/fphar.2014.00030

32. Herrmann TL, Morita CT, Lee K, Kusner DJ (2005) Calmodulin kinase II regulates the maturation and antigen presentation of human dendritic cells. J Leukoc Biol 78:1397–1407. https://doi.org/10.1189/jlb.0205105

33. Hoch B, Meyer R, Hetzer R, Krause EG, Karczewski P (1999) Oxidation of CaMKII and Thr-17 of phospholamban. J Mol Cell Cardiol 31:793–802. https://doi.org/10.1128/MCB.01117-10

34. Hudmon A, Schulman H, Hudmon A, Schulman H, Hudmon A, Schulman H, Hudmon A, Schulman H (2002) Neuronal Ca\(^{2+}\)/calmodulin-dependent protein kinase in failing and nonfailing human myocardium. Circ Res 84:713–721

35. Hudmon A, Schulman H (2002) Neuronal Ca\(^{2+}\)/calmodulin-dependent protein kinase II: the role of structure and autoregulation in cellular function. Annu Rev Biochem 71:473–510. https://doi.org/10.1146/annurev.biochem.71.110601.135410

36. Hudmon A, Schulman H (2002) Structure-function of the multifunctional Ca\(^{2+}\)/calmodulin-dependent protein kinase II. Biochem J 364:593–611. https://doi.org/10.1042/BJ20020228

37. Hudmon A, Schulman H, Kim J, Maltez JM, Pitt GS (2005) CaMKII tethers to L-type Ca\(^{2+}\) channels, establishing a local and dedicated integrator of Ca\(^{2+}\) signals for facilitation. J Cell Biol 171:537–547. https://doi.org/10.1083/jcb.200505155

38. Johnston AS, Lehnart SE, Burgoyne JR (2015) Ca\(^{2+}\)(2+) signaling in the myocardium by (red) regulation of PKA/CaMKII. Front Pharmacol 6:166. https://doi.org/10.3389/fphar.2015.00166

39. Joiner ML, Koval OM, Li J, He BJ, Allamargot C, Yang J, Guan X, Zimovskikh IM, Luczak ED, Hall DD, Fink BD, Chen B, Yang J, Moore SA, Scholz TD, Stace S, Mohler PJ, Sivitz WI, Song LS, Anderson ME (2012) CaMKII determines mitochondrial stress responses in heart. Nature 491:269–273. https://doi.org/10.1038/nature11444

40. Kahl CR, Means AR (2003) Regulation of cell cycle progression by calcium/calmodulin-dependent pathways. Endocr Rev 24:719–736. https://doi.org/10.1210/er.2003-0008

41. Kang PM, Izumo S (2000) Apoptosis and heart failure: a critical review of the literature. Circ Res 86:107–113

42. Kemi OJ, Ellingsen O, Ceci M, Grimvaldi S, Smith GL, Condorelli G, Wisloff U (2007) Aerobic interval training enhances cardiomyocyte contractility and Ca\(^{2+}\) cycling by phosphorylation of CaMKII and Thr-17 of phospholamban. J Mol Cell Cardiol 43:354–361. https://doi.org/10.1016/j.yjmcc.2007.06.013

43. Kirchhefer U, Schmitz W, Scholz H, Neumann J (1999) Activity of CaM-dependent protein kinase and Ca\(^{2+}\)/calmodulin-dependent protein kinase in failing and nonfailing human hearts. Cardiovasc Res 42:254–261

44. Kreusser MM, Lehmann LH, Keranov S, Hoting MO, Oehl U, Kohilhaas M, Reil JC, Neumann K, Schneider MD, Hill JA, Dobrev D, Maack C, Maier LS, Grone HJ, Katus HA, Olson EN, Backs J (2014) Cardiac CaMK kinase II genes delta and gamma contribute to adverse remodeling but redundantly inhibit calcineurin-induced myocardial hypertrophy. Circulation 130:1262–1273. https://doi.org/10.1161/CIRCULATIONAHA.114.006185

45. Kreusser MM, Lehmann LH, Wolf N, Keranov S, Jungmann A, Grone HJ, Muller OJ, Katus HA, Backs J (2016) Inducible cardiomyocyte-specific deletion of CaMK kinase II protects from pressure overload-induced heart failure. Basic Res Cardiol 111:65. https://doi.org/10.1007/s00395-016-0581-2

46. Kung G, Konstantinidis K, Kitisis RN (2011) Programmed necrosis, not apoptosis, in the heart. Circ Res 108:1017–1036. https://doi.org/10.1161/CIRCRESAHA.110.225730

47. Lai Y, Nairn AC, Gorelick F, Greengard P (1987) Ca\(^{2+}\)/calmodulin-dependent protein kinase II: identification of autophosphorylation sites responsible for generation of Ca\(^{2+}\)/calmodulin independence. Proc Natl Acad Sci USA 84:5710–5714

48. Li C, Cai X, Sun H, Bai T, Zheng X, Zhou XW, Chen X, Gill DL, Li J, Tang XD (2011) The delta isoform of calmodulin kinase II mediates pathological cardiac hypertrophy by interfering with the HDAC4–MEF2 signaling pathway. Biochem Biophys Res Commun 409:125–130. https://doi.org/10.1016/j.bbrc.2011.04.128

49. Ling H, Gray CB, Zambon AC, Grimm M, Gu Y, Dalton N, Purcell NH, Peterson K, Brown JH (2013) Ca\(^{2+}\)/calmodulin-dependent protein kinase II delta mediates myocardial ischemia/reperfusion injury through nuclear factor-kappaB. Circ Res 112:935–944. https://doi.org/10.1161/CIRCRESAHA.112.276915

50. Ling H, Zhang T, Pereira L, Means CK, Cheng H, Gu Y, Dalton ND, Peterson KL, Chen J, Bers D, Brown JH (2009) Requirement for Ca\(^{2+}\)/calmodulin-dependent protein kinase II in the transition from pressure overload-induced cardiac hypertrophy to heart failure in mice. J Clin Investig 119:1230–1240. https://doi.org/10.1172/JCI38022

51. Little GH, Saw A, Bai Y, Dow J, Marjomar P, Simkhovich B, Leeka J, Kedes L, Kloner RA, Poizat C (2009) Critical role of nuclear calcium/calmodulin-dependent protein kinase IIdeltaB in cardiomyocyte survival in cardiomyopathy. J Biol Chem 284:24857–24868. https://doi.org/10.1074/jbc.M109.003186

52. Liu X, Yao M, Li N, Wang C, Zheng Y, Cao X (2008) CaMKII promotes TLR-triggered proinflammatory cytokine and type I interferon production by directly binding and activating TAK1 and IRF3 in macrophages. Blood 112:4961–4970. https://doi.org/10.1182/blood-2008-03-144022

53. Machado FS, Souto JT, Rossi MA, Esper L, Tanowitz HB, Aliberti J, Silva JS (2008) Nitric oxide synthase-2 modulates TLR7-mediated type I interferon production by Trypanosoma cruzi-infected cardiac myocytes. Microbes Infect 10:1558–1566. https://doi.org/10.1016/j.micinf.2008.09.009

54. Maggioni AP, Dahlstrom U, Filippatos G, Chioncel O, Crespo Leiro M, Drozdz J, Fruhwald F, Gullestad L, Logeart D, Persson H, Ponikowski P, Rauchhaus M, Voors AA, Nielsen OW, Zannad F, Tavazzi L, Heart Failure Association of the European Society of C (2013) EUROSURVival Pilot Programme: regional differences and 1-year follow-up results of the Heart Failure Pilot
55. Maier LS, Bers DM (2007) Role of Ca\textsuperscript{2+}/calmodulin-dependent protein kinase (CaMK) in excitation-contraction coupling in the heart. Cardiovasc Res 73:631–640. https://doi.org/10.1016/j.cardiores.2006.11.005

56. Mann DL, McMurray JJ, Packer M, Swedberg K, Borer JS, Colucci WS, Dijan J, Drexlner H, Feldman A, Kober L, Krum H, Liu P, Nieminen M, Tavazzi L, van Veldhuisen DJ, Waldenstrom A, Warren M, Westheim A, Zannad F, Fleming T (2004) Targeted anticytokine therapy in patients with chronic heart failure: results of the Randomized Etanercept Worldwide Evaluation (RENEWAL). Circulation 109:1594–1602. https://doi.org/10.1161/01.CIR.0000144907.27666.B2

57. Marx SO, Marks AR (2013) Dysfunctional ryanodine receptors in the heart: new insights into complex cardiovascular diseases. J Mol Cell Cardiol 58:225–231. https://doi.org/10.1016/j.yjmcc.2013.03.005

58. Mattiazzi A, Bassani RA, Escobar AL, Palomeque J, Valverde Mattiazzi A, Kranias EG (2014) The role of CaMKII regulation of phospholamban activity in heart disease. Front Pharmacol 5:5. https://doi.org/10.3389/fphar.2014.00005

59. Mattiazzi A, Vittone L, Mundina-Weilnmann C (2007) Ca\textsuperscript{2+}/calmodulin-dependent protein kinase: a key component in the contractile recovery from acidosis. Cardiovasc Res 73:648–656. https://doi.org/10.1016/j.cardiores.2006.12.002

60. Meyer T, Hansom PI, Stryer L, Schulman H (1992) Calmodulin trapping by calcium–calmodulin-dependent protein kinase. Science 256:1199–1202

61. Mishra S, Gray CB, Miyamoto S, Bers DM, Brown JH (2011) Location matters: clarifying the concept of nuclear and cytosolic CaMKII subtypes. Circ Res 109:1354–1362. https://doi.org/10.1161/CIRCRESAHA.111.248401

62. Mustroph J, Neef S, Maier LS (2017) CaMKII as a target for arrhythmia suppression. Pharmacol Ther 176:22–31. https://doi.org/10.1016/j.pharmthera.2016.10.006

63. Mustroph J, Wagemann O, Lebik S, Tarnowski D, Ackermann J, Meyer T, Hanson PI, Stryer L, Schulman H (1992) Calcium/calmodulin-dependent protein kinase: a key component in the contractile recovery from acidosis. Cardiovasc Res 73:648–656. https://doi.org/10.1016/j.cardiores.2006.12.002

64. Meyer T, Hansom PI, Stryer L, Schulman H (1992) Calmodulin trapping by calcium–calmodulin-dependent protein kinase. Science 256:1199–1202

65. Mishra S, Gray CB, Miyamoto S, Bers DM, Brown JH (2011) Location matters: clarifying the concept of nuclear and cytosolic CaMKII subtypes. Circ Res 109:1354–1362. https://doi.org/10.1161/CIRCRESAHA.111.248401

66. Nogueira NP, de Souza CF, Saraiva FM, Sultano PE, Dalmau SR, Bruno RE, de Goncalves RL, Laranja GA, Leal LH, Coelho MG, Masuda CA, Oliveira MF, Paes MC (2011) Heme-induced ROS in Trypanosoma cruzi activates CaMKII-like that triggers epispermigote proliferation. One helpful effect of ROS. PLoS ONE 6:e25935. https://doi.org/10.1371/journal.pone.0025935

67. Oguma NP, de Souza CF, Saraiva FM, Sultano PE, Dalmau SR, Bruno RE, de Goncalves RL, Laranja GA, Leal LH, Coelho MG, Masuda CA, Oliveira MF, Paes MC (2011) Heme-induced ROS in Trypanosoma cruzi activates CaMKII-like that triggers epimastigote proliferation. One helpful effect of ROS. PLoS ONE 6:e25935. https://doi.org/10.1371/journal.pone.0025935

68. Noguera NP, de Souza CF, Saraiva FM, Sultano PE, Dalmau SR, Bruno RE, de Goncalves RL, Laranja GA, Leal LH, Coelho MG, Masuda CA, Oliveira MF, Paes MC (2011) Heme-induced ROS in Trypanosoma cruzi activates CaMKII-like that triggers epispermigote proliferation. One helpful effect of ROS. PLoS ONE 6:e25935. https://doi.org/10.1371/journal.pone.0025935

69. Ozcan L, Cristina de Souza J, Harari AA, Backs J, Olson EN, Tabas I (2013) Activation of calcium/calmodulin-dependent protein kinase II in obesity mediates suppression of hepatic insulin signaling. Cell Metab 18:803–815. https://doi.org/10.1016/j.cmet.2013.10.011

70. Ozcan L, Wong CC, Li G, Xu T, Pajvani U, Park SK, Wronska A, Chen BX, Marks AR, Fukamitsu A, Backs J, Singer HA, Yates JR 3rd, Accili D, Tabas I (2012) Calcium signaling through CaMKII regulates hepatic glucose production in fasting and obesity. Cell Metab 15:739–751. https://doi.org/10.1016/j.cmet.2012.03.002

71. Padfield JG, Jin JN, Koushiappi E, Mills NL, Robinson SD, Cruden NL, Lucking AJ, Chia S, Harding SA, Newby DE (2013) Cardiovascular effects of tumour necrosis factor alpha antagonism in patients with acute myocardial infarction: a first in human study. Heart 99:1330–1335. https://doi.org/10.1136/heartjnl-2013-303648

72. Passier R, Zeng H, Frey N, Naya FJ, Nicol RL, McKinsey TA, Overbeek P, Richardson JA, Grant SR, Olson EN (2000) CaM kinase signaling induces cardiac hypertrophy and activates the MEF2 transcription factor in vivo. J Clin Invest 105:1395–1406. https://doi.org/10.1172/JCI8551

73. Pellicena P, Schulman H (2014) CaMKII inhibitors: from research tools to therapeutic agents. Front Pharmacol 5:21. https://doi.org/10.3389/fphar.2014.00021

74. Peng W, Zhang Y, Zheng M, Cheng H, Zhu W, Cao CM, Xiao RP (2010) Cardioprotection by CaMKII-deltaB is mediated by phosphorylation of heat shock factor 1 and subsequent expression of inducible heat shock protein 70. Circ Res 106:110–119. https://doi.org/10.1161/CIRCRESAHA.109.210914

75. Pereira C, Schar DJ, Bachli EB, Kurzer MO, Schoedon G (2008) Wnt5A/CaMKII signaling contributes to the inflammatory response of macrophages and is a target for the antiinflammatory action of activated protein C and interleukin-10. Arterioscler Thromb Vasc Biol 28:504–510. https://doi.org/10.1161/ATVBAHA.107.157438

76. Perez-Molina JA, Molina I (2017) Chagas disease. Lancet. https://doi.org/10.1016/S0140-6736(17)31612-4

77. Pocock SJ, Ariti CA, McMurray JJ, Maggioni A, Kober L, Squire IB, Swedberg K, Dobson J, Poppe KK, Whalley GA, Meta-Analysis Global Group in Chronic Heart F, Doughty RN (2013) Predicting survival in heart failure: a risk score based on 39 372 patients from 30 studies. Eur Heart J 34:1404–1413. https://doi.org/10.1093/eurheartj/het237

78. Rellos P, Pike AC, Niesen FH, Salas E, Lee WH, von Delft F, Knapp S (2010) Structure of the CaMKIIdelta/calmodulin complex reveals the molecular mechanism of CaMKII kinase activation. PLoS Biol 8:e1000426. https://doi.org/10.1371/journal.pbio.1000426

79. Rose AJ, Frosig C, Kiens B, Wojtaszewski JF, Richter EA (2007) Effect of endurance exercise training on Ca\textsuperscript{2+} calmodulin-dependent protein kinase II expression and signalling in skeletal muscle of humans. J Physiol 583:785–795. https://doi.org/10.1113/jphysiol.2007.138529

80. Rose AJ, Hargreaves M (2003) Exercise increases Ca\textsuperscript{2+}–calmodulin-dependent protein kinase II activity in human skeletal muscle. J Physiol 553:303–309. https://doi.org/10.1113/jphysiol.2003.054171

81. Rosenberg OS, Deindl S, Sung RJ, Nairn AC, Kuriyan J (2005) Structure of the autoinhibited kinase domain of CaMKII and SAXS analysis of the holoenzyme. Cell 123:849–860. https://doi.org/10.1016/j.cell.2005.10.029

82. Sag CM, Kohler AC, Anderson ME, Backs J, Maier LS (2011) CaMKII-dependent SR Ca leak contributes to doxorubicin-induced impaired Ca handling in isolated cardiac myocytes. J Mol Cell Cardiol 51:749–759. https://doi.org/10.1016/j.yjmcc.2011.07.016
83. Sales Junior PA, Molina I, Fonseca Murta SM, Sanchez-Montalva A, Salvador F, de Oliveira RC, Carneiro CM (2017) Experimental and clinical treatment of Chagas disease: a review. Am J Trop Med Hyg. https://doi.org/10.4269/ajtmh.16-0761

84. Sanders PN, Koval OM, Jaffer OA, Prasad AM, Businga TR, Scott JA, Hayden PJ, Luczak ED, Dickey DD, Allamgorot C, Olivier AK, Meyerholz DK, Robison AJ, Winder DG, Blackwell TS, Dworski R, Sutterwala FS, Anderson ME, Grumbach IM (2010) CaMKII is essential for the proarrhythmic effects of oxidation. Sci Transl Med 5:195ra197. https://doi.org/10.1126/scitranslmed.3006135

85. Sato K, Wu T, Laham RJ, Johnson RB, Douglas P, Li J, Sellke FW, Bunting S, Simons M, Post MJ (2001) Efficacy of intracoronary or intravenous VEGF165 in a pig model of chronic myocardial ischemia. J Am Coll Cardiol 37:616–623

86. Sebag SC, Koval OM, Paschke JD, Winters CJ, Jaffer OA, LaPrade ML, Mundy-Weinberg A, Costa SC, Paes MC (2009) Heme-induced Calmodulin-dependent protein kinase II triggers cell membrane injury by inducing complement factor B gene expression after myocardial infarction. J Mol Cell Cardiol 52:1135–1144. https://doi.org/10.1016/j.yjmcc.2012.01.021

87. Simmerman HK, Collins JL, Theibert JL, Wegener AD, Jones LR (1986) Sequence analysis of phospholamban. Identification of phosphorylation sites and two major structural domains. J Biol Chem 261:13333–13341

88. Singer HA, Benscoter HA, Schworer CJ, Jaffer OA, Dworski R, Sutterwala FS, Anderson ME, Grumbach IM (2010) Mitochondrial CaMKII inhibition in airway epithelium protects against allergic asthma. JCI Insight 2:e88297. https://doi.org/10.1172/jci.insight.88297

89. Singh MV, Kapournis A, Amin AS, van der Made I, Auftiero S, Khan MA, Schumacher CA, Janssens KJ, van Spaendonck-Zwarts KY, Remme CA, Backs J, Verkerk AO, Baartsinke A, Pinto YM, Creemers EE (2018) RBM20 mutations induce an arrhythmogenic dilated cardiomyopathy related to disturbed calcium handling. Circulation. https://doi.org/10.1161/CIRCULATIONAHA.117.031947

90. Trachtenberg BH, Hare JM (2017) Inflammatory cardiomyopathic syndromes. Circ Res 121:803–818. https://doi.org/10.1161/CIRCRESAHA.117.302221

91. van den Hoogenhof MMG, Beqguali A, Amin AS, van der Made I, Auftiero S, Khan MA, Schumacher CA, Janssens KJ, van Spaendonck-Zwarts KY, Remme CA, Backs J, Verkerk AO, Baartsinke A, Pinto YM, Creemers EE (2018) RBM20 mutations induce an arrhythmogenic dilated cardiomyopathy related to disturbed calcium handling. Circulation. https://doi.org/10.1161/CIRCULATIONAHA.117.031947

92. van Oort RJ, McCauley MD, Dixit SS, Pereira L, Yang Y, Respress JL, Wang Q, De Almeida AC, Skapura DG, Anderson ME, Bers DM, Wehrens XH (2010) Ryanodine receptor phosphorylation by calcium/calmodulin-dependent protein kinase II promotes life-threatening ventricular arrhythmias in mice with heart failure. Circulation 122:2669–2679. https://doi.org/10.1161/CIRCULATIONAHA.110.982298

93. Vila-Petroff M, Salas MA, Said M, Valverde CA, Sapia L, Portianni F, Hajar RJ, Kraaineg M, Widom-Meierlen C, Mattiazi A (2007) Calmodulin Kinase II inhibition protects against necrosis and apoptosis in irreversible ischemia–reperfusion injury. Cardiovasc Res 73:689–698. https://doi.org/10.1161/j.cirdiores.2006.12.003

94. Wehrens XH, Lehnaart RE, Reiken SR, Marks AR (2004) Ca2+/calmodulin-dependent protein kinase II phosphorylation regulates the cardiac ryanodine receptor. Circ Res 94:e61–e70. https://doi.org/10.1161/01.RES.0000125626.33738.E2

95. Weinreuter M, Kreussler MM, Beckendorf J, Schreiter FC, Leuschner F, Lehnart SE, Reiken SR, Marks AR (2004) Ca2+/calmodulin-dependent protein kinase II phosphorylation regulates the cardiac ryanodine receptor. Circ Res 94:e61–e70. https://doi.org/10.1161/01.RES.0000125626.33738.E2

96. Westra J, Brouwer E, van Roosmalen JA, Doornbos-van der Meer AO, Baartscheer A, Pinto YM, Creemers EE (2018) RBM20 mutations induce an arrhythmogenic dilated cardiomyopathy related to disturbed calcium handling. Circulation. https://doi.org/10.1161/CIRCULATIONAHA.117.031947

97. Swirski FK, Nahrendorf M (2013) Leukocyte behavior in atherosclerosis, myocardial infarction, and heart failure. Science 339:161–166. https://doi.org/10.1126/science.1230719

98. Tombs RM, Faison MO, Turbeville JM (2003) Organization and evolution of multifunctional Ca(2+)/CaM-dependent protein kinase genes. Gene 322:17–31

99. Toussaint F, Charbel C, Allen BG, Ledoux J (2016) Vascular CaMKII: heart and brain in your arteries. Am J Physiol Cell Physiol 311:C462–C478. https://doi.org/10.1152/ajpcell.00341.2015

100. Vila-Petroff M, Salas MA, Said M, Valverde CA, Sapia L, Portianni F, Hajar RJ, Kraaineg M, Widom-Meierlen C, Mattiazi A (2007) Calmodulin Kinase II inhibition protects against necrosis and apoptosis in irreversible ischemia–reperfusion injury. Cardiovasc Res 73:689–698. https://doi.org/10.1161/j.cirdiores.2006.12.003

101. Wehrens XH, Lehnaart RE, Reiken SR, Marks AR (2004) Ca2+/calmodulin-dependent protein kinase II phosphorylation regulates the cardiac ryanodine receptor. Circ Res 94:e61–e70. https://doi.org/10.1161/01.RES.0000125626.33738.E2

102. van Oort RJ, McCauley MD, Dixit SS, Pereira L, Yang Y, Respress JL, Wang Q, De Almeida AC, Skapura DG, Anderson ME, Bers DM, Wehrens XH (2010) Ryanodine receptor phosphorylation by calcium/calmodulin-dependent protein kinase II promotes life-threatening ventricular arrhythmias in mice with heart failure. Circulation 122:2669–2679. https://doi.org/10.1161/CIRCULATIONAHA.110.982298

103. Vila-Petroff M, Salas MA, Said M, Valverde CA, Sapia L, Portianni F, Hajar RJ, Kraaineg M, Widom-Meierlen C, Mattiazi A (2007) Calmodulin Kinase II inhibition protects against necrosis and apoptosis in irreversible ischemia–reperfusion injury. Cardiovasc Res 73:689–698. https://doi.org/10.1161/j.cirdiores.2006.12.003

104. Wehrens XH, Lehnart SE, Reiken SR, Marks AR (2004) Ca2+/calmodulin-dependent protein kinase II phosphorylation regulates the cardiac ryanodine receptor. Circ Res 94:e61–e70. https://doi.org/10.1161/j.cirdiores.2006.12.003

105. Westra J, Brouwer E, van Roosmalen JA, Doornbos-van der Meer AO, Baartscheer A, Pinto YM, Creemers EE (2018) RBM20 mutations induce an arrhythmogenic dilated cardiomyopathy related to disturbed calcium handling. Circulation. https://doi.org/10.1161/CIRCULATIONAHA.117.031947

106. Westra J, Brouwer E, van Roosmalen JA, Doornbos-van der Meer AO, Baartscheer A, Pinto YM, Creemers EE (2018) RBM20 mutations induce an arrhythmogenic dilated cardiomyopathy related to disturbed calcium handling. Circulation. https://doi.org/10.1161/CIRCULATIONAHA.117.031947

107. Wight SC, Schellenberger U, Ji L, Wang H, Larrick JW (1997) Calmodulin-dependent protein kinase II mediates signal transduction in apoptosis. FASEB J 11:843–849

108. Wu Y, Gao Z, Chen B, Koval OM, Singh MV, Guan X, Hundi TJ, Kutschke W, Sarma S, Grumbach IM, Wehrens XH, Mohler PJ, Song LS, Anderson ME (2009) Calmodulin kinase II is required for fight or flight sinoatrial node physiology. Proc Natl Acad Sci USA 106:1231–1245. https://doi.org/10.1073/pnas.0806422106

109. Wu Y, Valdivia HH, Wehrens XH, Anderson ME (2016) A single protein kinase C or calmodulin kinase II site does not control the cardiac pacemaker Ca(2+) clock. Circ Arrhythm Electrophysiol 9:e003180. https://doi.org/10.1161/CIRCEP.115.003180
111. Xu X, Yang D, Ding JH, Wang W, Chu PH, Dalton ND, Wang HY, Birmingham JR Jr, Ye Z, Liu F, Rosenfeld MG, Manley JL, Ross J Jr, Chen J, Xiao RP, Cheng H, Fu XD (2005) ASF/SF2-regulated CaMKIIdelta alternative splicing temporally reprograms excitation–contraction coupling in cardiac muscle. Cell 120:59–72. https://doi.org/10.1016/j.cell.2004.11.036

112. Yang Y, Zhu WZ, Joiner ML, Zhang R, Oddis CV, Hou Y, Yang J, Price EE, Gleaves L, Eren M, Ni G, Vaughan DE, Xiao RP, Anderson ME (2006) Calmodulin kinase II inhibition protects against myocardial cell apoptosis in vivo. Am J Physiol Heart Circ Physiol 291:H3065–H3075. https://doi.org/10.1152/ajpheart.00353.2006

113. Zhang R, Khoo MS, Wu Y, Yang Y, Grueter CE, Ni G, Price EE Jr, Thiel W, Guatimosim S, Song LS, Madu EC, Shah AN, Vishnivetskaya TA, Atkinson JB, Gurevich VV, Salama G, Lederer WJ, Colbran RJ, Anderson ME (2005) Calmodulin kinase II inhibition protects against structural heart disease. Nat Med 11:409–417. https://doi.org/10.1038/nm1215

114. Zhang T, Brown JH (2004) Role of Ca2+/calmodulin-dependent protein kinase II in cardiac hypertrophy and heart failure. Cardiovasc Res 63:476–486. https://doi.org/10.1016/j.cardiores.2004.04.026

115. Zhang T, Johnson EN, Gu Y, Morissette MR, Sah VP, Gigena MS, Belke DD, Dillmann WH, Rogers TB, Schulman H, Ross J Jr, Brown JH (2002) The cardiac-specific nuclear delta(B) isoform of Ca2+/calmodulin-dependent protein kinase II induces hypertrophy and dilated cardiomyopathy associated with increased protein phosphatase 2A activity. J Biol Chem 277:1261–1267. https://doi.org/10.1074/jbc.M108525200

116. Zhang T, Kohlhaas M, Backs J, Mishra S, Phillips W, Dybkova N, Chang S, Ling H, Bers DM, Maier LS, Olson EN, Brown JH (2007) CaMKIIdelta isoforms differentially affect calcium handling but similarly regulate HDAC/MEF2 transcriptional responses. J Biol Chem 282:35078–35087. https://doi.org/10.1074/jbc.M707083200

117. Zhang T, Maier LS, Dalton ND, Miyamoto S, Ross J Jr, Bers DM, Brown JH (2003) The deltaC isoform of CaMKII is activated in cardiac hypertrophy and induces dilated cardiomyopathy and heart failure. Circ Res 92:912–919. https://doi.org/10.1161/01.RES.0000069686.31472.C5

118. Zhang T, Zhang Y, Cui M, Jin L, Wang Y, Lv F, Liu Y, Zheng W, Shang H, Zhang J, Zhang M, Wu H, Guo J, Zhang X, Hu X, Cao CM, Xiao RP (2016) CaMKII is a RIP3 substrate mediating ischemia- and oxidative stress-induced myocardial necroptosis. Nat Med 22:175–182. https://doi.org/10.1038/nm.4017

119. Zhu WZ, Wang SQ, Chakir K, Yang D, Zhang T, Brown JH, Devic E, Kobilka BK, Cheng H, Xiao RP (2003) Linkage of beta1-adrenergic stimulation to apoptotic heart cell death through protein kinase A-independent activation of Ca2+/calmodulin kinase II. J Clin Invest 111:617–625. https://doi.org/10.1172/JCI16326