Chapter 24
The Role of CaM Kinase II in Cardiac Function in Health and Disease

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Abstract Ca^{2+}-calmodulin-dependent protein kinase II (CaMKII) has emerged as a critical molecule involved in the regulation of cell processes that are dependent on calcium, including excitation–contraction coupling (ECC), cell growth, and death. In addition to a generally accepted signaling pathway through beta-adrenergic receptors (ARs), oxidative stress has been suggested to promote CaMKII activation. Since many cardiac diseases, including those characterized by a phenomenon called as ischemia/reperfusion injury (IRI), are associated with oxidative stress, CaMKII is likely to be a crucial molecule underlying the phenotypes of this cardiac injury. In contrast, there is also evidence that CaMKII activation leading to phosphorylation of phospholamban and the subsequent decrease of calcium overload is important for attenuation of post-ischemic cardiac contracture, indicating that CaMKII may act as a double-edged sword depending on the actual conditions. In addition, CaMKII over-activation has been shown to destabilize the action potential and trigger early and delayed afterdepolarizations promoting arrhythmias. Experimental studies from our laboratory have revealed that CaMKII inhibition does not protect the heart against all types of IRI-induced ventricular arrhythmias, but it is capable to reduce the occurrence of the most life-threatening tachyarrhythmias. Moreover, the CaMKII inhibition appears to reduce oxidative stress and thus to increase the viability of cardiomyocytes upon IRI. In this manuscript, a dual role of CaMKII in IRI is reviewed and beneficial effects of the CaMKII inhibition are discussed with studies that have shown the opposite results.

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We conclude that CaMKII activation either inhibition should be carefully considered in effort to mitigate cardiac IRI-induced injury.

Keywords Calcium/calmodulin-dependent protein kinase · Ischemia–reperfusion injury · Arrhythmias · Contractile dysfunction · Cell death · Oxidative stress

24.1 Introduction

In spite of considerable effort undertaken so far, exact mechanisms of the development of phenotypes of myocardial ischemia/reperfusion injury (IRI), such as arrhythmias, contractile dysfunction, and cell death, are not still satisfactorily elucidated. Impaired calcium homeostasis has emerged as a crucial event initiating IR-induced cardiac dysfunction and hence, many calcium handling proteins have been suggested to be a potential target to mitigate the outcomes of this type of cardiac injury. Recently, a role of calcium/calmodulin-dependent protein kinase (CaMKII), a protein kinase regulating intracellular calcium levels and activated by calcium itself, has raised interest of many investigators. In the heart, CaMKII, a multimer consisting of 8–12 subunits, is expressed in two isoforms, such as the delta and gamma (CaMKIIδ, CaMKIIγ); however, the CaMKIIδ is the predominant one [1]. CaMKIIδ has different splice variants, which are localized in the nucleus (δ_B) and the cytoplasm (δ_C), suggesting that different cellular processes can be regulated by this protein kinase. The cytoplasmic isoform regulates many of the key proteins of excitation–contraction coupling (ECC), while the nuclear isoform is likely to be important for the activation of various transcriptional factors regulating cellular growth and death [1, 2]. In addition, the expression of the splice variants of CaMKIIδ is age dependent. In fact, δ_B predominates in the adult heart, while δ_C is abundant in the embryonic heart [2].

24.2 Activation of CaMKIIδ in Physiological and Pathological Conditions

Under resting conditions CaMKIIδ is inactive; however, once the levels of intracellular Ca^{2+} are increased and Ca^{2+} is bound to calmodulin to form Ca^{2+}/CaM complex, which is subsequently bound to the regulatory domain of the kinase, the autoinhibitory effect of the domain is released and the kinase becomes activated. In conditions characterized by the sustained formation of Ca^{2+}/CaM, the catalytic domain of the kinase can activate a neighboring subunit at threonine 286/287 ($Thr^{286/287}$). This process referred to as autophosphorylation results in the persistent activation of CaMKIIδ, indicating that conditions that promote autophosphorylation increase CaMKII activation and thus may change the

physiological action of the kinase into the pathological effect [3]. One of the mechanisms leading to the significant elevation of intracellular Ca^{2+} is the stimulation of the beta-adrenergic receptors (ARs) that result in the activation of protein kinase A and subsequently in Ca^{2+} influx through the L-type calcium channels (LTCC) [4]. As the levels of catecholamines are increased in ischemic tissue, persistently elevated beta-ARs stimulation during IRI favors CaMKII autophosphorylation. Phosphorylation of CaMKII itself at $Thr^{286/287}$ has several important implications, such as the increase in the affinity of Ca^{2+}/CaM binding termed "CaM trapping", and maintaining catalytic activity of the kinase even in the absence of CaM binding [5, 6]. On the other hand, the phosphorylation of CaMKII at Thr^{306} prevents Ca^{2+}/CaM binding that results in the decreased CaMKII activity. This type of autophophosphorylation of CaMKII is considered to be the self-inhibitory effect, which provides a negative feedback regulation of the kinase activity, in particular, under resting/basal intracellular Ca^{2+} levels [7]. Another potential mechanism, which is assumed to participate in CaMKII activation during IRI is the production of reactive oxygen species (ROS). It has been shown that in the presence of oxidative stress, a pair of methionine residues within the regulator domain ($M^{281/282}$) become oxidized that results in the allosterical rearrangement of the domains of the kinase and consequently into the initiation/promotion of the Ca^{2+}/CaM-independent activation of CaMKII [8]. ROS leading to CaMKII activation have been found to be generated by NADPH oxidase upon the stimulation of angiotensin II [9]. Recently, Wagner et al. [10] have demonstrated that free calcium and a functional sarcoplasmic reticulum (SR) are required for ROS activation of CaMKII and that ROS-activated CaMKIIδ further enhances late Na current (I_{Na}), which in turn may lead to cellular Na^+ and Ca^{2+} overload [10]. Consequently, CaMKII-dependent changes in late I_{Na} have been suggested to be a major contributor to cellular arrhythmias [11]. In line, I_{Na} inhibition has been found to reverse arrhythmias in a transgenic Ca^{2+}/calmodulin-dependent protein kinase IIδ_C mice developing heart failure [12]. Hence, it appears that functional consequences of CaMKII activation under pro-oxidant conditions are initially dependent on Ca^{2+} content, but later on they may be propagated due to oxidative stress rather than due to Ca^{2+} overload (Fig. 24.1). This may also be of relevance in conditions associated with IRI, where ROS production is enhanced, indicating a link between oxidative stress and CaMKII-mediated cardiac injury.

24.3 Proteins Phosphorylated by CaMKIIδ

Early studies have revealed that the CaMKIIδ$_C$ phosphorylates and thus activates LTCC, both the predominant pore-forming subunit LTCCα ($Ca_v1.2$) and the β-subunit ($Ca_v1.3$) and thus mediates gradation of I_{Ca}, a process termed I_{Ca} facilitation [13–15]. CaMKII phosphorylation sites are presently unknown on the LTCCα subunit, while the phosporylation of Thr 498 on the LTCCβ subunit is necessary for increases in LTCC opening and dynamic facilitation responses in
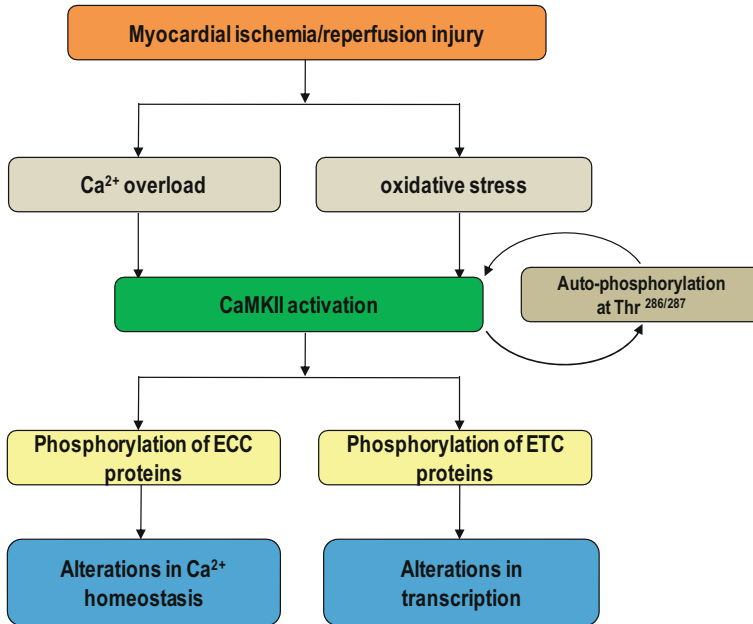
Fig. 24.1 Proposed myocardial ischemia/reperfusion-induced pathways leading to CaMKII activation, subsequent increase in phosphorylation of target proteins and its consequences. *ECC* excitation–contraction coupling, *ETC* excitation-transcription coupling, *Thr*[286/287] phosphorylation at the site of threonine 286/287

cardiomyocytes [13]. It is known that I_{Ca} in addition to increase in Na current increases net inward currents during the action potential plateau resulting in the action potential duration (APD) prolongation that is observed on electrocardiogram as the long QT interval. As consequence of excessive APD prolongation, the heart becomes more prone to the development of early afterdepolarizations (EADs) and delayed afterdepolarizations (DADs) that further give raise to various types of arrhythmias, including atrial fibrillation and ventricular tachyarrhythmias [16]. It has been shown that the CaMKII-induced hyperphosphorylation of the LTCC and of the ryanodine receptor (RyR), which induces Ca^{2+} leak from the SR and subsequently a net inward Na current via NCX, is associated with the induction of EADs and DADs, respectively [17, 18]. Association of CaMKII activation with APD prolongation and induction of EADs has also been shown upon the administration of clofilium, a K^+ channel antagonist drug that is along with other drugs of class III anti-arrhythmic class known to reduce repolarising currents [19].

Other Ca^{2+}-handling protein activated by CaMKII is the ryanodine receptor that upon Ca^{2+} entry through LTCC mediates release of Ca^{2+} from the SR. This process is referred to as Ca^{2+}-induced Ca^{2+} release. Although Ser^{2809} and Ser^{2815} have been shown to be phosphorylated by CaMKII, additional phosphorylation sites of

the RyR cannot be ruled out [20]. CaMKII action on Ca^{2+} release via the RyR is controversial. Cellular studies have revealed that the hyperphosphorylation of the RyR results in inappropriate diastolic Ca^{2+} release from the SR and thus contributes to impaired cardiac contractility and promotion of DADs. In mice, in which the S^{2814} Ca^{2+}/calmodulin-dependent protein kinase II site on RyR2 is constitutively activated, pathological SR Ca^{2+} release has been found to contribute to fatal pacing-induced arrhythmias, while genetic ablation of the CaMKII site on RyR2 protected mutant mice from arrhythmias [21]. These findings have supported an important role of CaMKII-mediated activation of RyR in arrhythmogenesis and sudden cardiac death. In addition to the increase, the decrease of Ca^{2+} release from the SR as a consequence of CaMKII overactivation has been shown [22, 23], supporting the view that CaMKII has a regulatory action on the RyR. This further seems to be determined by the presence/absence of the pathological state. Whether it is a CaMKII stimulatory or inhibitory action on the RyR function, and in which particular conditions, remains to be investigated in detail. In contrast to the above discussed Ca^{2+} handling proteins, which increase the intracellular Ca^{2+} levels, the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) acts oppositely and removes cytoplasmic Ca^{2+}. In humans, SERCA is responsible for about 70 % of cytoplasmic Ca^{2+} removal, while the remaining Ca^{2+} content is removed by the sodium/calcium exchanger (NCX) [24]. Under resting conditions, SERCA is inhibited by phospholamban (PLN) and its phosphorylation at Ser^{16} or at Thr^{17}, which is regulated by protein kinase A and CaMKII, respectively, relieves this inhibitory action. As consequence, this phosphorylation accelerates Ca^{2+} removal from the cytoplasm, and subsequently increases the SR Ca^{2+} content [25, 26]. With respect to SERCA regulation of frequency-dependent acceleration of relaxation (FDAR) determined by the Ca^{2+} removal, it has been shown that CaMKII is an important mediator of this property of cardiomyocytes, but it is unknown which key proteins underlie this process. It has been suggested that the modulatory effects of CaMKII on the PLN and secondary on the SERCA are more important in conditions characterized by slower, irregular frequencies, while they are likely to be less active under physiological, high steady-state frequencies [27].

24.4 Role of CaMKII in the Development of Phenotypes of Myocardial Ischemia/Reperfusion Injury

24.4.1 CaMKII as a Critical Component of Cascades Leading to IRI-Induced Ventricular Arrhythmias and Contractile Dysfunction

Based on findings that CaMKII overexpression and/or activity are increased in a mouse model of failing heart, which is known to have proarrhythmic remodeling, action potential prolongation, besides impaired contractile function, hypertrophy

and left ventricle chamber dilatation, CaMKII has been suggested to induce/promote electrical instability of the heart [28, 29]. Likewise, in the other mouse model of hypertrophied heart with the CaMKIV overexpression, which was associated with the increased CaMKII activity, the APD prolongation and the induction of EADs have been recorded [18]. In addition to these animal models, CaMKII has also been shown to be a proarrhythmic molecule in a model of catecholaminergic polymorphic ventricular tachycardia (CPVT). In this inherited arrhythmogenic disease characterized by the cardiac RyR2 mutation, which leads to abnormal diastolic Ca^{2+} leak from the SR upon adrenergic stimulation, CaMKII has been suggested to play an important role in the generation of the fatal arrhythmias [30]. CaMKII-dependent phosphorylation of the RyR, resulting in Ca^{2+} leak from the SR, has also been suggested to underlie mechanisms of digitalis-induced arrhythmias [31]. However, the CaMKII-mediated genesis of ventricular arrhythmias in conditions of IRI, which differ in several cellular and molecular mechanisms from all above-mentioned types of arrhythmias, is less known. We have clearly shown that CaMKII is involved in the genesis of R-induced ventricular arrhythmias. Although KN-93, an inhibitor which binds to the regulatory domain of the CaMKII, did not show any capability to modulate the incidence and duration of ventricular tachycardia, it protected the heart against the most life-threatening arrhythmia—ventricular fibrillation (VF) (Fig. 24.2a, b). Further analysis of the genesis of VF has revealed that the mean number and the duration of a single episode of VF were significantly decreased in the KN-93-treated group (Fig. 24.2c, d). In accordance with the study of Bell et al. [32], only one of eight KN-93 treated hearts exhibited ventricular tachycardia and/or fibrillation. Although in that study and ours, KN-93 was used to study CaMKII implication in IRI-induced arrhythmogenesis, the dose of the CaMKII inhibitor was different. In our studies, we use a dose of 0.5 μmol/l that is the fivefold lower than the dose used in other laboratories [32–34]. Pedersen et al. [35] have shown that increased CaMKII activity induces arrhythmias during metabolic acidification and that this effect is associated with spontaneous Ca^{2+} waves, which in turn are abolished by KN-93. Since acidosis is present during IRI, it is likely that this mechanism underlies the genesis of IRI-initiated arrhythmias. Interestingly, in our recent study, we have shown that the increased expression of the total CaMKIIδ in the hearts subjected to IRI co-exist with the higher NCX1 content, while there is such no co-existence with the expression of LTCCα [36]. These findings suggest that alterations in LTCCα protein content are unlikely to participate in CaMKII-depedendent phenotypes of heart injury, including arrhythmias. Thus, it can by hypothesized that the higher protein content of CaMKIIδ may trigger arrhythmias in conditions of IRI due to the increased NCX1-dependent promotion of DADs rather than due to LTCCα-induced EADs. Of note, the beta subunit of LTCC (LTCCβ) has been suggested to be crucial for CaMKII signaling promoting EADs and cardiomyopathy [37]. Said et al. [38] have suggested that CaMKII-dependent phosphorylation of SR proteins, in particular Ser^{2814} on RyR2 and Thr^{17} on PLN contribute to reperfusion-induced arrhythmias. Furthermore, in that study, no detectable EADs in transgenic mice with targeted inhibition of CaMKII at the
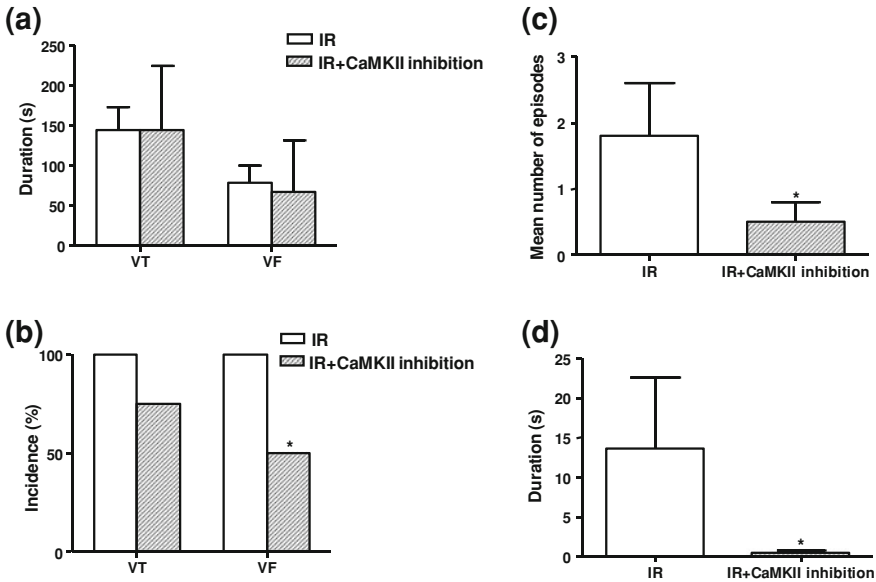
Fig. 24.2 Influence of CaMKII inhibition on reperfusion-induced ventricular arrhythmias after 30 min global ischemia in the isolated rat hearts. **a** The incidence and **b** total duration of ventricular tachyarrhythmias (ventricular tachycardia and fibrillation). **c** Mean number of episodes and **d** duration of a single episode of ventricular fibrillation (B). *P < 0.05 vs. non-treated control hearts

level of cardiac SR membranes, while there was the genesis of EADs-induced premature ventricular beats in wild type mice [38].

In addition to disturbances in cardiac rhythm associated with the increased CaMKII expression and/or activity, a role of the kinase in the development of contractile dysfunction, the other phenotype of IRI, has been extensively studied. Although excitation and contraction are a complex, CaMKII action in these two processes can produce the different effects that are independent of either one. In fact, Bell et al. [32] have reported that CaMKII mitigates R-induced ventricular tachyarrhythmias, however, this inhibition diminished contractile performance of the intact heart in the initial post-ischemic period. Moreover, CaMKII inhibition produced negative inotropic effects and increased coronary flow [32]. In contrast, in our recent study, we did not show these unwanted effects of KN-93 on coronary flow or cardiac contractility under baseline conditions. It is noteworthy that any effects of the drug observed during stabilization period can influence the final outcomes of IRI. In our hands, KN-93 administered 10 min prior to induction of I changed neither left ventricular systolic and end-diastolic pressure, nor maximal rates of pressure development and fall as the indexes of contraction and relaxation. On the other hand, CaMKII inhibition improved post-ischemic recovery of contractile function and attenuated diastolic contracture evidenced by a smaller rise of left ventricular end-diastolic pressure [36]. We further analyzed an overall heart

Fig. 24.3 Influence of CaMKII inhibition on the post-ischemic recovery of the pressure-rate product (*PRP*) after 30 min global ischemia and 40 min of reperfusion in isolated rat hearts. *P < 0.05 vs. non-treated control hearts
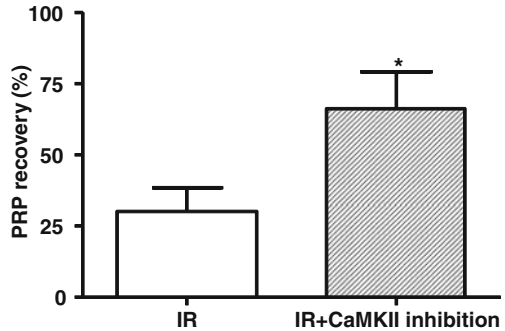
performance that in the Langendorff-perfused heart is determined as the pressure-rate product (PRP; LVDP x HR). Its better recovery in the KN-93-treated hearts also confirmed that CaMKII negatively affects contractile function of the heart subjected to IRI (Fig. 24.3).

24.4.2 CaMKII as a Mediator of Cellular Death Induced by Ischemia and Reperfusion Injury

It is generally known that the increased intracellular Ca^{2+} levels induce, besides disturbances in ECC, apoptotic and necrotic cell death. The two types of IRI-induced cell death are distinct. Necrotic cell death is characterized by membrane disruption, cell swelling, lysis, and inflammatory response, while apoptosis results in DNA fragmentation, prevents inflammation, and preserves membrane integrity [39]. Some studies have suggested that programmed death is induced by ischemia in the absence of reperfusion [40, 41], while other studies have demonstrated that reperfusion accelerates apoptosis initiated during ischemia [40, 42]. In addition, it has been reported that apoptosis is triggered during reperfusion and does not manifest during ischemia [43]. In the pathogenesis of programmed death, the mitochondria plays an important role, from which pro-apoptotic markers are released through the mitochondrial permeability transition pore (mPTP) and the mitochondrial apoptosis channel (mAC, or Bax channel). Their opening is dependent on the intracellular calcium levels; mAC/Bax channel may open in cells that are unloading calcium, while conditions associated with calcium overload cause and sustain mPTP opening [44, 45]. Of note, cells subjected to sustained mPTP opening are destined to die by necrosis; however, cells in which mPTP reverses before out-membrane rupture could either avoid necrosis and die by apoptosis or survive [46]. mPTP forming and opening is chiefly regulated by Bcl-2 family proteins; predominance of anti-apoptotic markers (Bcl-2, Bcl-xL) over the apoptotic ones (Bax, Bad) preserves mPTP opening and release of cytochrome C into the cytoplasm, where it is bound with ATP and caspase-9 to form apoptosome [47]. This complex further activates caspase-3 to

drive the execution phase of apoptosis. Since CaMKII regulates the cytoplasmic Ca^{2+} levels, it has been hypothesized that it is somehow involved in signaling pathway leading to IRI-induced cell death. Indeed, it has been shown that the mitigation of Ca^{2+} overload induced by the stimulation of beta-ARs is dependent on the CaMKII-mediated phosphorylation of PLN, thereby implicating PLN as an important regulator of anti-apoptotic action of CaMKII inhibition [17]. In our study, we showed that protein content of cytochrome C, caspase-9, and pro-caspase-3 were decreased by the CaMKII inhibitor (Fig. 24.4a–c). In addition, the pharmacological inhibition of CaMKII by KN-93 decreased the content of pro-apoptotic protein Bax and increased the levels of anti-apoptotic Bcl-2 as well as Bcl-2/Bax ratio (Fig. 24.4d–f). In line, Vila-Petroff et al. [34] have shown that the presence of KN-93 and the CaMKII-inhibitory peptide (AIP) in the perfusion medium are capable to decrease the extent of TUNEL-positive cells, and caspase-3 activity in the hearts subjected to IRI. Furthermore, the findings of that study established a cascade of CaMKII-dependent events integrating the NCX, the SR, and mitochondria that promote intrinsic cellular death. Likewise, Salas et al. [33] have confirmed that CaMKII is involved in the intrinsic apoptotic cellular death and showed that CaMKII does not participate in the extrinsic cascade of apoptosis. Both these studies have also shown that CaMKII is implicated into the regulation of necrotic death; CaMKII inhibition reduced the release of lactate dehydrogenase (LDH), a marker of necrosis [33, 34]. NCX in apoptosis in the hearts subjected to I/R also seems to play a role in our study; indeed, we showed that the increased NCX protein content in the ischemic hearts was downregulated by KN-93 [36]. However, it should be pointed out that the study of Salas et al. [33], Vila-Petroff et al. [34], and ours differ in some very important aspects. First, we used a protocol of reversible IRI, while those scientific groups induced irreversible IRI with a long-term R phase (120 min). Second, in our study, a dose of KN-93 used to inhibit CaMKII was fivefold lower than in the above-cited studies [33, 34]. Of note, higher doses of KN-93 has been reported to inhibit not only CaMKII but also other protein kinases such as, protein kinase C, A, and myosin light chain kinase [48].

Since it is known that oxidative stress is associated with CaMKII activation, and plays a crucial role in the pathogenesis of IRI as well as in the induction of intrinsic cellular death [49, 50], we analyzed protein content of pro- and anti-oxidant enzymes. Although the expression of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase), which generates superoxide, was increased in the ischemic hearts, CaMKII inhibition normalized these changes (Fig. 24.5a). On the other hand, the protein levels of superoxide dismutase (SOD), which catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide, was not changed by the CaMKII inhibitor (Fig. 24.5b). Hydrogen peroxide per se is one of the main contributors of oxidative stress; however, by the action of the other anti-oxidant enzyme, catalase, it is converted into water and oxygen and thereby the damaging effects of hydrogen peroxide are ameliorated. In our hands, the protein content of catalase did not differ among the groups (Fig. 24.5c). Of note, SOD and CAT treatment of isolated hearts has been found to prevent the IRI-induced
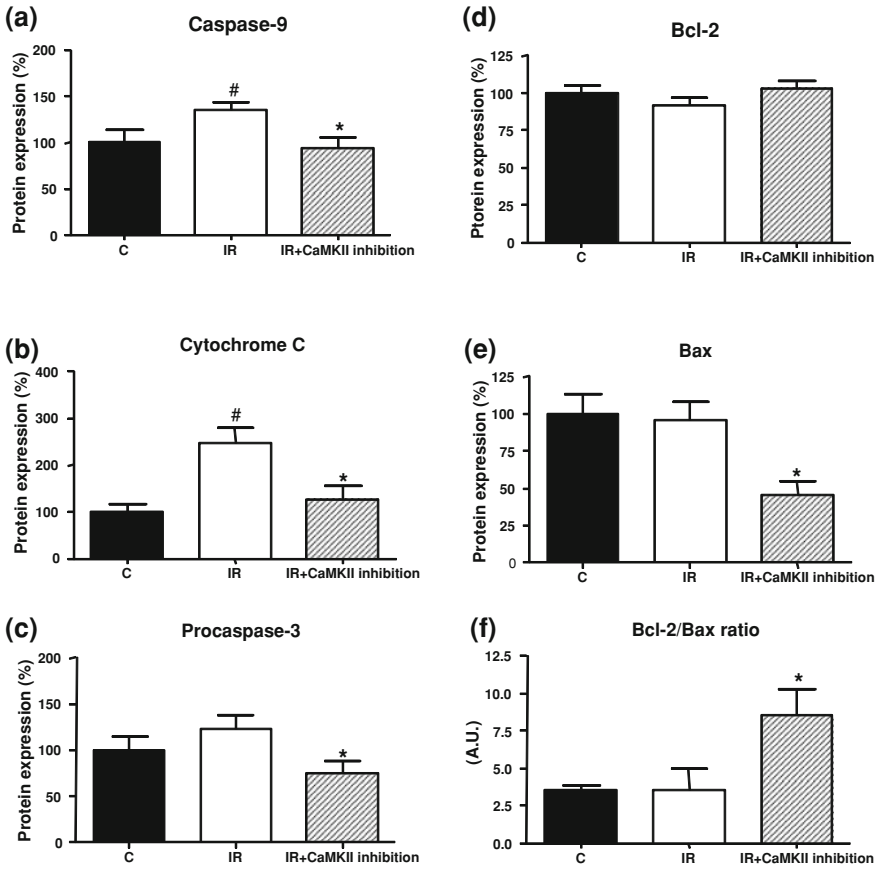
Fig. 24.4 Influence of CaMKII inhibition on extent of apoptosis in the hearts subjected to reversible ischemia/reperfusion injury. Protein content of **a** caspase-9, **b** cytochrome C, **c** procaspase-3, **d** bax, **e** bcl-2, and **f** bcl-2/bax ratio. *P < 0.05 vs. non-treated ischemic/reperfused hearts, #P < 0.05 vs. baseline pre-ischemic state

alterations in CaMKII phosphorylation of SERCA2a, PLN, and RyR and recover the depressed myocardial function in the hearts subjected to IRI [51, 52]. These findings have confirmed a proposed link between OS-mediated cardiac injury and CaMKII activation.

24.5 Conclusions

From the foregoing discussion it is apparent that CaMKII activation during IRI exhibits rather deleterious than beneficial effects. First, CaMKII is likely to be one of the molecules, at least partially, responsible for the proarrhythmic consequences

Fig. 24.5 Influence of CaMKII inhibition on protein content of the enzymes regulating oxidative stress in the hearts subjected to reversible ischemia/ reperfusion injury. a NADPH oxidase, b superoxide dismutase (SOD), and c catalase (CAT). *$P < 0.05$ vs. non-treated ischemic/ reperfused hearts, #$P < 0.05$ vs. baseline pre-ischemic state
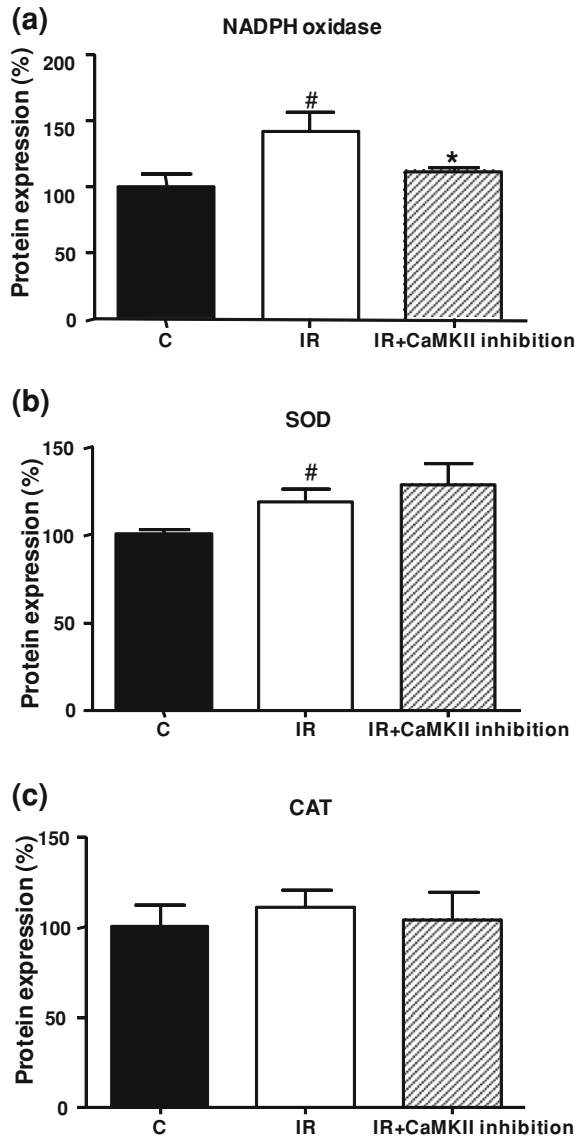
of excessive QT interval prolongation. Second, due to Ca^{2+} mishandling it can participate in post-ischemic cardiac contracture. Third, it mediates the alterations in Ca^{2+}-dependent cascades that induce/promote cell death. And last but not the least, it is apparent that oxidative stress has an important role in all these CaMKII-dependent phenotypes of IRI. Based on this, strategies for CaMKII inhibition may represent an effective tool to protect the heart against injury and sudden cardiac death. On the other hand, as it has already been mentioned, Bell et al. [32] have
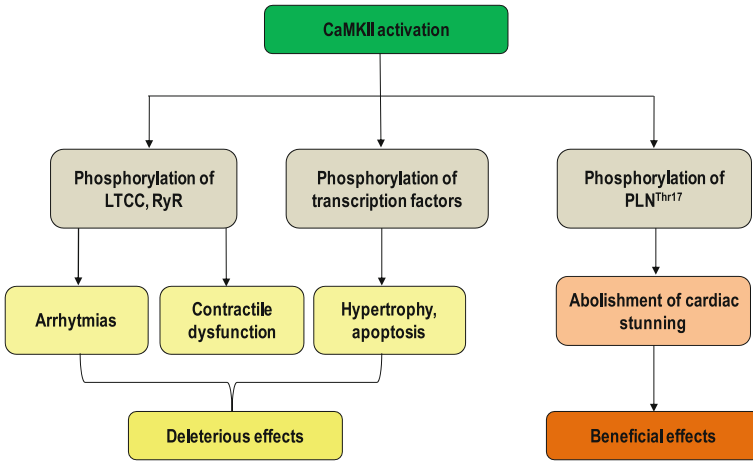
Fig. 24.6 CaMKII as a double-edged sword producing either beneficial or deleterious effects depending on the the conditions of myocardial ischemia/reperfusion injury. *LTCC* L-type calcium channel, *RyR* ryanodine receptors, *PLN* phospholamban

reported that CaMKII inhibition diminishes cardiac stunning of the intact heart in the initial post-ischemic period suggesting that CaMKII activation is important for the abolishment of cardiac contracture. Protective role of CaMKII activity on contractile function abolished by IRI has been suggested to be mainly mediated by the phosphorylation of the PLN. It has been shown that phosphorylation of the Thr^{17} site of the PLN at the onset of R is important for amelioration of Ca^{2+}-mishandling and recovery of cardiac function in the stunned heart [38, 53]. In line, Osada et al. [54] have shown that the IRI-induced depression in Ca^{2+}/calmodulin-dependent SR Ca^{2+}-uptake activity and the IRI-induced decrease in the CaMKII activity can be prevented by preconditioning (PC), whereas KN-93 blocks these effects of PC, suggesting that CaMKII plays an important role in PC-mediated cardio protection. Based on these reports, it is apparent that CaMKII activation in settings of IRI may also exhibit beneficial effects. Taken together, CaMKII may play a dual role in conditions of IRI and thus it may be termed as a double-edged sword (Fig. 24.6). Taken together, we believe that whether CaMKII activation is beneficial or deleterious in conditions of IRI should also be based on consideration of protocol details. It refers mainly to the duration of ischemia, and the dose of a CaMKII inhibitor. The negative effects of CaMKII overactivation have been observed in the case of long-term ischemia, while beneficial effects of CaMKII activation, which were associated with the phosphorylation of PLN, have been reported in a study with a short ischemic insult. The choice of dose of KN-93, which can directly influence a wide variety of target proteins, not only CaMKII alone, is the other point that needs to be taken into consideration while discussing the beneficial or deleterious effects of CaMKII activation. Although it is evident that alterations in CaMKII expression/activity produce direct/indirect changes in ECC during IRI, the exact mechanisms involved are still less known. It is

presumable that the consequences of CaMKII overactivation/expression are mainly linked with Ca^{2+}-mishandling; however, it cannot be ruled out that other proteins may also be influenced. In fact, there are some indications that CaMKII targets also K^+, Na^+ channels [55, 56]. Furthermore, utilizing more selective CaMKII inhibitors, such as CaMKII autoinhibitory peptide, can help to understand better the role of CaMKII in the heart subjected to IRI. Finally, we believe that CaMKII is a potential therapeutic target; however, in order to achieve protection of the heart against injury either by CaMKII inhibition or activation many aspects should be carefully considered.

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