Calmodulin kinase II inhibition protects against myocardial cell apoptosis in vivo

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myocardial infarction; isoproterenol; programmed cell death; phospholamban

CALCIUM IS A UBQUITOUS SIGNAL for regulating cellular functions, including survival and death (4). The multifunctional Ca2+/calmodulin-dependent protein kinase II (CaMKII) is dynamically activated by cellular Ca2+ (10) and serves as a downstream signal transduction molecule for many conditions marked by Ca2+ mobilization, including myocardial infarction (MI) and activation of the β-adrenergic receptor (β-AR) pathway by isoproterenol (Iso) (40). Left ventricular (LV) dilation and dysfunction are two elements that define an adverse remodeling process that is associated with cardiomyocyte death and increased mortality in patients with structural heart disease (18). CaMKII inhibition reduces adverse LV remodeling after Iso and MI in part by improving the mechanical properties of surviving ventricular myocytes (40), but it is unknown whether CaMKII inhibition can also contribute salutary effects in clinically relevant disease models by reducing cell death.

Recently, CaMKII was identified to be a facilitator of cardiomyocyte apoptosis in response to Iso in vitro (43). The antiapoptotic actions of CaMKII inhibition in vitro were mirrored by sarcoplasmic reticulum (SR) Ca2+ depletion using thapsigargin, a potent antagonist of the SR Ca2+-ATPase (SERCA2a) (43). These findings suggest that CaMKII might also be important for regulating apoptosis in vivo under conditions of increased β-AR stimulation. However, the relationship of in vitro-to-in vivo findings for CaMKII signaling in apoptosis is far from certain, because of the vastly different conditions of intracellular Ca2+ in cultured cells compared with the beating heart. We used a mouse model with cardiomyocyte-delimited transgenic expression of a CaMKII inhibitory peptide (AC3-I, I is for inhibitor) to test the potential role of CaMKII in apoptosis in vivo. A second line of transgenic mice expressed an inactive, scrambled version of AC3-I (AC3-C, C is for control). AC3-C mice and wild-type (WT) littermates were used as controls. Myocardial function was equivalent in AC3-I, AC3-C, and WT hearts and isolated ventricular myocytes at baseline and after Iso (40). Apoptosis was not evident above control levels in AC3-I mice after Iso, whereas AC3-C and WT mice exhibited significant increases above control levels in response to Iso injection, indicating that the previously established proapoptotic role of CaMKII in isolated cells was also evident in vivo.

MI is the most common cause of adverse LV remodeling, heart failure, and excessive mortality in patients with structural heart disease (18). MI and heart failure are marked by neurohumoral activation (3), upregulation of CaMKII (17), and increased apoptosis (25). Previous cellular studies showing that CaMKII inhibition reduced catecholamine-triggered apoptosis (43) suggested that CaMKII inhibition might also be effective...
in reducing apoptosis during MI. We took advantage of our in vivo model to study the effects of CaMKII inhibition on apoptosis after MI. We studied AC3-I, AC3-C, and WT mice after surgical MI to test whether CaMKII was a significant regulator of apoptosis during infarction. Apoptosis was significantly reduced in AC3-I compared with AC3-C and WT mice.

Phospholamban (PLN) is a negative regulator of cytoplasmic Ca\(^{2+}\) uptake into the SR and is a phosphorylation target for CaMKII (5). PLN phosphorylation by CaMKII and SR Ca\(^{2+}\) content are significantly reduced in AC3-I transgenic mouse ventricular myocytes (40), whereas cardiomyocyte SR Ca\(^{2+}\) content is increased independently of CaMKII inhibition in PLN\(^{-/-}\) mice (35). Taken together, these findings suggest the hypothesis that PLN is an important molecular determinant for the antiapoptotic actions of CaMKII inhibition. On the other hand, PLN\(^{-/-}\) mice are a model for enhancing SR Ca\(^{2+}\) and myocardial function, and PLN ablation can improve adverse LV remodeling in some mouse models of cardiomyopathy (23, 29), suggesting that PLN\(^{-/-}\) mice do not have enhanced apoptosis at baseline. We found that PLN\(^{-/-}\) mice did not have increased apoptosis at baseline but showed greatly enhanced susceptibility to apoptosis induced by Iso, compared with WT type littermate controls. Iso-stimulated apoptosis in PLN\(^{-/-}\) hearts was due to increased Ca\(^{2+}\) because it was prevented by verapamil pretreatment. These results support a strong association between PLN, myocyte Ca\(^{2+}\), and apoptosis in vivo while suggesting that therapeutic strategies for increasing SR Ca\(^{2+}\) uptake may also increase vulnerability to apoptosis.

To test whether CaMKII inhibition was antiapoptotic in the absence of PLN, we interbred AC3-I and AC3-C transgenic mice with PLN\(^{-/-}\) mice and subjected these mice to MI and Iso stimulation. Conventional pharmacological tools for controlling SR Ca\(^{2+}\), such as ryanodine and thapsigargin, are incompatible with in vivo studies. One advantage of our genetic strategy is that PLN ablation eliminates differences in SR Ca\(^{2+}\) content between AC3-I and AC3-C-expressing hearts by increasing SR Ca\(^{2+}\) content to an equivalent level (35). CaMKII inhibition failed to reduce apoptosis after MI in the absence of PLN, whereas a protective effect of CaMKII inhibition persisted after Iso, albeit with a trend (\(P = 0.07\)) toward increased Iso-dependent apoptosis in AC3-I-expressing mice with PLN ablation compared with AC3-I-expressing mice with normal PLN expression. These findings show that CaMKII is a downstream signal for apoptosis during \(\beta\)-AR stimulation and MI and suggest that PLN is an important molecular target for the antiapoptotic actions of CaMKII inhibition in the beating heart.

**MATERIALS AND METHODS**

**Transgenic mice.** Transgenic mice with CaMKII inhibition (AC3-I) and transgenic control mice (AC3-C) were generated by myocardial-targeted overexpression of mini-genes encoding AC3-I or AC3-C, as described (40). For some experiments, we bred AC3-I and AC3-C mice into a PLN\(^{-/-}\) background (19) and used the fifth to seventh generations of male and female PLN\(^{-/-}\), AC3-I \(\times\) PLN\(^{-/-}\), and AC3-C \(\times\) PLN\(^{-/-}\) mice (8–12 wk of age) for this study. All experiments were approved by Vanderbilt University and University of Iowa Animal Care Committees.

**Iso treatment.** A single high dose of Iso (150 mg/kg ip) or an identical volume of saline as a vehicle control was injected into mice. Verapamil (50 mg/kg ip) was given 15 min before Iso for some studies. Transverse LV sections from three to eight hearts in each experimental group were used for histological assays, as previously described (40).

**MI.** MI surgery was performed on mechanically ventilated (tidal volume 0.5 ml, 120 breaths/min), anesthetized mice (33 \(\mu\)g/g ip pentobarbital), as previously described (40). Sections 1 mm below the ligation suture line were used for apoptosis measurements. Sham MI surgery omitted the coronary artery ligation but was otherwise similar to the MI surgery. After surgery, the animals were extubated from mechanical ventilation and allowed to recover for 2–5 h before they were killed.

**Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling staining.** Mice were killed by cervical dislocation. We weighed the mouse and rapidly exposed the heart. While the heart was beating in situ, we injected 5,000 U of heparin and 1 ml of 2 M KCl into the right ventricle to prevent coagulation and to arrest the heart in diastole. We then perfused the heart with 20 ml of PBS and 20 ml of 10% formalin by injection through the right ventricle to wash out blood cells. The heart was then fixed in 10% formalin for 24 h before being rinsed, frozen, embedded, and sectioned at 7-µm thickness. The serial tissue sections from the LV equator region were used for apoptosis measurement by using terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay. We performed TUNEL assays using In Situ Cell Death Detection Kit (Roche), according to the manufacturer’s instructions. Tissue sections were permeated with proteinase K (20 µg/ml in 10 mM Tris-HCl, pH 7.4) for 20 min (37°C) and stained with labeling solution (including rhodamine-labeled 2-deoxyuridine 5’-triphosphate and DNA polymerase). We pretreated the tissue sections with DNase I (100 U/ml) for 15 min for positive controls, whereas DNA polymerase was omitted for negative controls. 4,6-Diamidino-2-phenylindole (1 µg/ml, Vector) was used for nuclear staining, and myocytes were identified by expression of enhanced green fluorescent protein (eGFP) (40). We confirmed that the measured TUNEL-positive cells were cardiomyocytes by only counting TUNEL-stained nuclei encompassed by eGFP. Tissue sections were examined with an epifluorescent microscope (Olympus BX41). In Iso- or saline-treated hearts, a mid-LV cross section was divided into quarters, and the number of apoptotic myocytes in each quarter was counted under \(\times400\) magnification. After MI, the apoptosis rate was much higher, so nine random fields (\(\times200\) magnification) within the ischemic areas were examined, and the myocyte apoptosis rate was reported as the average number of apoptotic myocytes per high power field. Only cells with positive TUNEL-stained nuclei that were clearly identified as myocytes were counted and expressed as the fraction of the approximate number of cells per field. Investigators were blinded to the treatment and genetic identity of the mice in all measurements.

**DNA laddering.** DNA laddering was performed on hearts after MI surgery, where the burden of apoptosis was large compared with hearts from Iso-treated mice. Hearts were minced, washed three times with PBS, and frozen in liquid nitrogen. Aliquots were thawed in 720 µl of lysis buffer (10 mM Tris-HCl, pH 8.0, 100 mM NaCl, 25 mM EDTA, and 0.5% SDS) and quickly homogenized on ice using a glass-Teflon homogenizer. The tissue was digested with proteinase K at a final concentration of 1.0 mg/ml at 37°C overnight and then incubated with DNA-free RNase at 37°C for an additional hour. Sodium chloride was added to a final concentration of 1.3 M from a 5 M stock, and precipitate was removed by high-speed centrifugation. The supernatant was then extracted with 1.0 ml of 3 M NaCl-Tris-EDTA (TE; pH 8.0)-equilibrated phenol-chloroform-isoamyl alcohol (25:24:1), reextracted with chloroform-isoamyl alcohol alone, and then precipitated overnight in 2 vol of ethanol. The resulting DNA was washed in 70% ethanol and dissolved in TE (pH 8.0). After quantification with a spectrophotometer, 5 µg of DNA were loaded into 1.8% agarose gel containing 0.5 g/ml ethidium bromide. DNA electrophoresis was carried out in Tris-Borate-EDTA buffer at 80 V for 1–2 h. DNA ladder formation was visualized under ultraviolet
light (8). Investigators were blinded to the treatment and genetic identity of the mice in all measurements.

Echocardiography. Echocardiography was performed using a 15-MHz high-frequency transducer (Sonos 5500 Agilent) in unanesthetized mice as previously described (28).

Statistics. The null hypothesis was evaluated with Student’s t-test or ANOVA with \( \alpha = 0.05 \), as appropriate. All values are expressed as means ± SE.

RESULTS

CaMKII inhibition reduces Iso-stimulated cardiomyocyte apoptosis. Catecholamines can increase cellular Ca\(^{2+}\) and CaMKII activity (40), leading to myocyte apoptosis in vitro (43). Catecholamines are increased in vivo during ischemia (31) and heart failure and critically drive adverse clinical outcomes under these conditions (3). We treated AC3-I, AC3-C, and WT mice with Iso or saline injection to test whether CaMKII inhibition protects against catecholamine-induced myocyte apoptosis in vivo. Our previous studies showed that the \( \beta \)-AR density and the downstream protein kinase A activity, measured as Ser16 PLN phosphorylation, were equivalent in AC3-I, AC3-C, and WT mouse hearts (40). Thus differences in Iso response between AC3-I and control mice are due to reduced CaMKII activity.

Hearts from AC3-I mice treated with a single dose of Iso were significantly \( (P = 0.017) \) resistant to apoptosis compared with WT and AC3-C controls (Fig. 1), as assayed by TUNEL staining. Apoptosis was not different between saline-treated AC3-I, AC3-C, or WT mice (Fig. 1). The overall rate of apoptosis was low with this protocol, perhaps accounting for the lack of significant difference in TUNEL-positive staining between saline and Iso treatment for AC3-I, AC3-C, or WT mice. These findings indicate that CaMKII inhibition can significantly protect against, or even prevent, Iso-induced myocyte apoptosis in vivo, consistent with in vitro data (43).

CaMKII inhibition reduces cardiomyocyte apoptosis in ischemia. Intracellular Ca\(^{2+}\) overload contributes to myocyte death in ischemia, and, early in MI, apoptosis is a major mode of myocyte death (12). CaMKII is a downstream signal for cellular calcium change (7); however, the potential effect of CaMKII inhibition on apoptosis in ischemia is unknown. To test the potential role of CaMKII in ischemia-induced myocyte apoptosis in vivo, we surgically ligated the left coronary artery in AC3-I and AC3-C mice. Myocyte apoptosis was significantly lower \( (P = 0.018) \) in AC3-I hearts compared with AC3-C hearts 2 h after MI (Fig. 2, A and B), whereas sham surgery failed to elicit TUNEL-positive staining in AC3-I (n =...
5) or AC3-C (n = 5) mice (data not shown). We performed DNA laddering assays, which complemented and confirmed the results of TUNEL staining by showing reduced apoptosis in AC3-I hearts after MI surgery (Fig. 2A).

Surgical MI ultimately causes cell death of all cardiomyocytes dependent on the ligated artery, as evidenced by our previous report that AC3-I, AC3-C, and WT mice had similar areas of scar formation 3 wk after MI surgery (40). To test whether CaMKII inhibition could protect against apoptosis later than 2 h after coronary artery ligation, we repeated TUNEL staining measurements 5 h after MI surgery. CaMKII inhibition continued to significantly (*P < 0.002) protect myocytes from apoptosis 5 h after MI compared with AC3-C or WT controls (Fig. 3). These findings show that CaMKII inhibition in vivo significantly inhibits or delays ischemia-induced myocyte apoptosis for at least 5 h, a time window with potentially important relevance for patients with acute coronary artery occlusion (15).

Our previous work showed that the size of the surgical infarction is large and consistently reproducible (40). To validate these previous results and test whether the apparent beneficial effects of CaMKII inhibition could be an artifact of inconsistency of the MI surgery, we used the percent area of TUNEL staining in LV transverse sections as an index of the ischemic burden in each of the treatment groups. Although the density of TUNEL staining was less in AC3-I compared with AC3-C and WT hearts (Figs. 2 and 3), the cross-sectional area with identifiable TUNEL staining was not different between AC3-I, AC3-C, and WT hearts (Fig. 4), suggesting that the ischemic burden was similar in all treatment groups.

PLN ablation increases β-AR-stimulated apoptosis by a Ca2+-dependent mechanism. PLN is a phosphorylation target for CaMKII, and the AC3-I mice have significantly reduced CaMKII-dependent PLN phosphorylation and reduced SR Ca2+ content (40). On the other hand, PLN−/− mice have elevated SR Ca2+ load (35) and significantly increased apoptosis after Iso (P < 0.001), compared with WT littermates (Fig. 5). Excess apoptosis in PLN−/− mice was due to intracellular Ca2+ overload because it was prevented by pretreatment with verapamil (Fig. 5). PLN−/− mice did not have more apoptosis than WT littermates under control (i.e., saline treatment) conditions (Fig. 5). These findings show that PLN ablation significantly increased myocardial susceptibility to apoptosis during β-AR stimulation without affecting basal apoptosis rates, and they raise the question of whether PLN is important for the antiapoptotic actions of CaMKII inhibition in response to Iso.

PLN−/− mice with CaMKII inhibition. To test whether CaMKII inhibition can protect against the enhanced susceptibility for Ca2+-dependent myocardial apoptosis seen in PLN−/− mice, we interbred AC3-I and AC3-C mice with PLN−/− mice (Fig. 6). The SR Ca2+ content of ventricular myocytes from these interbred mice was previously shown to be increased to an equal level in AC3-I- and AC3-C-expressing cells (35), suggesting that residual effects of CaMKII inhibition in AC3-I-expressing mice are independent of reduced SR Ca2+ content. The AC3-I × PLN−/− mice had slower heart rates compared with AC3-C × PLN−/− mice, consistent with the known effects of CaMKII inhibition to reduce cardiac pacemaker activity in vitro (33) and in vivo (40). The LV fractional shortening was modestly but significantly less in AC3
Fig. 3. CaMKII inhibition protects against apoptosis 5 h after MI surgery. A: photomicrographs from AC3-C and AC3-I LV transverse sections, arranged as in Fig. 2, 5 h after MI surgery. B: summary data are from 3–5 hearts/group and show a significant reduction in TUNEL-positive staining in AC3-I hearts compared with AC3-C and WT hearts (†P = 0.002).

Fig. 4. Equivalent ischemic areas in AC3-I, AC3-C, and WT hearts after MI surgery, as assessed by TUNEL staining. LV cross sections show TUNEL staining in AC3-I (A) and AC3-C (B) hearts after MI surgery. Yellow lines demarcate TUNEL-positive ischemic area from nonischemic tissue. Calibration bar indicates 1 mm. C: area of TUNEL-positive staining was measured in 3–5 hearts/group and expressed as a percentage of total area 5 h after MI surgery. There were no differences in percent area with TUNEL-positive staining between any of the groups.
AC3-I × PLN−/− compared with AC3-C × PLN−/− mice (Fig. 6, A–C), possibly due to slower heart rates in AC3-I-expressing mice. Neither the AC3-I × PLN−/− nor the AC3-C × PLN−/− mice showed evidence of cellular or cardiac hypertrophy (Fig. 6, D and E), cardiac fibrosis, or increased TUNEL staining (not shown) at baseline compared with WT littermates, indicating that interbred PLN−/− mice do not have overt myocardial disease. This is consistent with our finding that PLN−/− mice do not have increased apoptosis at baseline, compared with WT controls (Fig. 5).

**Apoptosis in PLN−/− mice with CaMKII inhibition.** CaMKII inhibition significantly protected against apoptosis after Iso even in the absence of PLN because AC3-I × PLN−/− mice had significantly less apoptosis (P < 0.001) than AC3-C × PLN−/− mice (Fig. 7). However, AC3-I × PLN+/− mice had a trend (P = 0.07) toward greater apoptosis with Iso than did AC3-I mice with PLN expression (compare Fig. 7B and Fig. 1B), suggesting that PLN contributes to the antiapoptotic mechanism of CaMKII inhibition. Control experiments with saline showed no difference (P = 0.6) in myocyte apoptosis in AC3-I × PLN−/− and AC3-C × PLN−/− hearts (Fig. 7). These findings show that CaMKII inhibition can reduce apoptosis during β-AR stimulation even in the absence of PLN, but they leave open the possibility that PLN...
participates in the antiapoptotic effects of myocardial CaMKII inhibition.

In contrast to the persistent protective effects of CaMKII inhibition on Iso-mediated apoptosis in the absence of PLN, the protective effect of CaMKII inhibition on apoptosis after MI was eliminated in PLN-/- hearts (Fig. 8, A and B). There was no difference in the surgically induced ischemic areas between AC3-I × PLN-/- and AC3-C × PLN-/- mice (Fig. 8D). These findings suggest that PLN is important for the antiapoptotic actions of CaMKII inhibition during ischemia.

**DISCUSSION**

Our results add to a growing body of evidence that CaMKII can be recruited as a pathological signal in heart (1, 41). Catecholamine-stimulated Ca2+ overload is a cause of cardiomyocyte death, but diverse signaling pathways potentially respond to changes in cellular Ca2+ (37). Recently, in vitro studies showed that CaMKII can play a critical role in SR Ca2+ overload-related myocyte apoptosis induced by Iso treatment (43). Our study extends these earlier findings in cultured cardiomyocytes by showing that CaMKII inhibition reduces ischemia-induced and prevents catecholamine-induced myocyte apoptosis in vivo, indicating that CaMKII is an important pathophysiological mediator of apoptosis. Our findings suggest that improved cardiac function originally reported in AC3-I mice after excessive Iso stimulation and MI (40) is, in part, due to reduced apoptosis. These results further suggest that CaMKII inhibition may be an important therapeutic approach for reducing myocyte loss in ischemia and structural heart disease. Cultured cell systems offer significant experimental advantages but exist under starkly different conditions than do working myocytes in situ. Our findings provide a new rationale for utilizing both in vitro and in vivo approaches in studies of CaMKII signaling, because CaMKII inhibition was successful in preventing or reducing apoptosis in response to Iso in vivo as well as in cultured adult ventricular myocytes (43).

MI is a major cause of structural heart disease and mortality worldwide (18). Currently, the most effective treatments to
reduce cell death and adverse remodeling from MI involve acute restoration of coronary blood flow within 6 h of occlusion (6). Thus interventions that effectively delay or reduce myocyte loss early in MI have the potential to improve the efficacy of reperfusion therapies by lengthening the time window for successful reperfusion and increasing the number of surviving cardiomyocytes. Our finding that CaMKII inhibition significantly delays myocyte apoptosis during MI, in a time frame that is important in patients, suggests that CaMKII inhibition could improve currently available therapies for myocardial ischemia. It will be important to repeat these experiments in larger animals with heart rates that are more similar to that of humans to understand whether the present findings are truly relevant to patients. A lack of highly specific CaMKII inhibitory drugs was one difficulty that motivated us to make transgenic mice with myocardial CaMKII inhibition (1). However, it will be necessary to develop CaMKII inhibitory drugs with improved specificity to perform studies in larger animals where genetic approaches to modulating CaMKII are less tractable than in mice.

Overload of intracellular Ca$^{2+}$ endoplasmic reticulum stores is an important stimulus for apoptosis in nonmuscle cells (9), but the potential relationship between SR Ca$^{2+}$ overload in cardiomyocytes and apoptosis is incompletely understood, in part because previous work has not evaluated these cellular components under physiological in vivo conditions. Our finding that PLN ablation enhances susceptibility to apoptosis and verapamil eliminates this susceptibility indicates that SR Ca$^{2+}$ overload contributes to β-AR-mediated apoptosis in the working heart. CaMKII inhibition significantly protects against SR Ca$^{2+}$ overload (40), whereas PLN ablation eliminates differences in SR Ca$^{2+}$ content between AC3-I- and AC3-C-expressing hearts (35). PLN ablation appears to reduce the beneficial effects of CaMKII inhibition on apoptosis during

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Fig. 7. PLN-independent protection from Iso-mediated apoptosis by CaMKII inhibition. A: photomicrographs show reduced TUNEL-positive staining in AC3-I × PLN$^{-/-}$ compared with AC3-C × PLN$^{-/-}$ LV transverse sections 2 h after Iso injection. Calibration bar indicates 15 μm. B: summary data for TUNEL-positive staining in Iso-treated hearts (6–7 hearts/group) and saline-treated hearts (3–4 hearts/group). Iso significantly (†P < 0.001) increased TUNEL staining in AC3-C × PLN$^{-/-}$ hearts compared with saline, whereas the AC3-I × PLN$^{-/-}$ hearts had less TUNEL staining than did the AC3-C × PLN$^{-/-}$ hearts (*P = 0.05).
β-AR stimulation and prevents protection of CaMKII inhibition against apoptosis during MI.

A potentially important implication of our findings is that efforts to target repletion of SR calcium content to improve mechanical function during heart failure may increase apoptosis. One characteristic change in failing human and animal myocytes is reduced SR calcium content that can contribute to depressed myocyte contractility (26, 27). Reduced SR Ca\(^{2+}\) is thus a potential target for therapy by inotropic drugs, by PLN knockout, or by SERCA2a overexpression. Although some studies have shown promise (14, 23, 29), others have shown worsened cardiomyopathy (16, 30, 32) by PLN ablation. A human mutation producing PLN that is incapable of negatively regulating SERCA2a results in severe cardiomyopathy and heart failure (16), suggesting that SR Ca\(^{2+}\) repletion could have negative consequences in patients. Our findings that PLN ablation can increase apoptosis during ISO provide a potential mechanism for understanding adverse outcomes related to therapeutic repletion of SR Ca\(^{2+}\) content.

The mechanisms of Ca\(^{2+}\) enhancement in MI and during isoproterenol are likely to be different. For example, the Na\(^{+}/Ca\(^{2+}\) exchanger is implicated in Ca\(^{2+}\) overload during MI (22), whereas increased L-type Ca\(^{2+}\) current may be preeminent during catecholamine stimulation (39). Thus it will be important for future studies to consider how differences in these and other cellular Ca\(^{2+}\) mobilizing mechanisms may affect CaMKII signaling. CaMKII inhibition reduces apoptosis 2 h (Fig. 2) and 5 h (Fig. 3) after MI and preserves LV function from 1 to 3 wk after MI (40). These findings support the idea that CaMKII inhibition could be a novel and effective therapy during ischemia. On the other hand, CaMKII activity may be beneficial for acute recovery of contractile function in ischemia-reperfusion by PLN phosphorylation (24). Furthermore, CaMKII activity may have physiological importance for controlling dynamic mechanical responses in heart (11). At present, it is unknown whether the early recovery of cellular mechanical function is beneficial or detrimental to cell survival.
Myocardial responses to CaMKII are highly dependent on the duration of increased CaMKII activity or CaMKII inhibition. CaMKII inhibition reduces L-type Ca\(^{2+}\) channel facilitation within minutes (2, 36, 38), and the addition of activated CaMKII to excised cardiomyocyte membranes induces L-type Ca\(^{2+}\) channels to enter a highly active gating mode within seconds (13). However, CaMKII inhibition in AC3-I mice does not impair basal contractile function or acute responses to Iso in isolated ventricular myocytes compared with AC3-C and WT cells (40), suggesting that chronic CaMKII inhibition (i.e., from the time of birth) does not significantly affect acute “fight or flight” responses to catecholamine stimulation in the heart. In contrast, CaMKII activation is required for maintaining normal cardiomyocyte contraction under conditions of prolonged (24 h) catecholamine exposure (34), suggesting that CaMKII can be critical for physiological stress responses in the heart. This concept is supported by findings that CaMKII overexpression (24–48 h) increases L-type Ca\(^{2+}\) current and enhances Ca\(^{2+}\) release from the SR (20). Truly chronic myocardial CaMKII overexpression, genetically induced from the time of birth, recapitulates important aspects of structural heart disease, including cardiomyocyte hypertrophy, premature death, and reduced contractile function (21, 42). Our findings that CaMKII is important for signaling apoptosis within 2 h after Iso and 2–5 h after MI suggest that “early” CaMKII activation may be important for initiating adverse LV remodeling responses that lead to heart failure and sudden death.

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