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Calmodulin Kinase II and Arrhythmias in a Mouse Model of Cardiac Hypertrophy

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Background—Calmodulin kinase (CaMK) II is linked to arrhythmia mechanisms in cellular models where repolarization is prolonged. CaMKII upregulation and prolonged repolarization are general features of cardiomyopathy, but the role of CaMKII in arrhythmias in cardiomyopathy is unknown.

Methods and Results—We studied a mouse model of cardiac hypertrophy attributable to transgenic (TG) overexpression of a constitutively active form of CaMKIV that also has increased endogenous CaMKII activity. ECG-telemetered TG mice had significantly more arrhythmias than wild-type (WT) littermate controls at baseline, and arrhythmias were additionally increased by isoproterenol. Arrhythmias were significantly suppressed by an inhibitory agent targeting endogenous CaMKII. TG mice had longer QT intervals and action potential durations than WT mice, and TG cardiomyocytes had frequent early afterdepolarizations (EADs), a hypothesized mechanism for triggering arrhythmias. EADs were absent in WT cells before and after isoproterenol, whereas EAD frequency was unaffected by isoproterenol in TG mice. L-type Ca^{2+} channels (LTCCs) can activate EADs, and LTCC opening probability (P_o) was significantly higher in TG than WT cardiomyocytes before and after isoproterenol. A CaMKII inhibitory peptide equalized TG and WT LTCC P_o and eliminated EADs, whereas a peptide antagonist of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger current, also hypothesized to support EADs, was ineffective.

Conclusions—These findings support the hypothesis that CaMKII is a proarrhythmic signaling molecule in cardiac hypertrophy in vivo. Cellular studies point to EADs as a triggering mechanism for arrhythmias but suggest that the increase in arrhythmias after β -adrenergic stimulation is independent of enhanced EAD frequency. (*Circulation*. 2002; 106:1288-1293.)

Key Words: arrhythmia ■ calcium ■ signal transduction

Cardiac arrhythmias are a leading cause of death in patients with cardiac hypertrophy, but identification of molecular signaling pathways linking cardiac hypertrophy to disordered electrical excitability has been lacking. Calmodulin kinase (CaMK) types II and IV are both present in the nucleus,¹ where they activate signaling pathways for cardiac hypertrophy,²⁻⁴ but CaMKII is also a proarrhythmic signaling molecule in cellular models of drug-induced action potential prolongation.^{5,6} Upregulation of CaMKII activity and expression seems to be a general feature of cardiomyopathy from diverse causes in patients⁷ and animal models,^{8,9} suggesting the hypothesis that CaMKII is a proarrhythmic signaling molecule in cardiomyopathy where cardiac repolarization is prolonged because of electrical remodeling.

See p 1180

Reduction in repolarizing outward K^+ currents and action potential and QT interval prolongation are also consistent findings in electrical remodeling in cardiac hypertrophy. However, the electrical remodeling phenotype, per se, does not explain the mechanism of arrhythmias in cardiac hypertrophy. On the other hand, L-type Ca^{2+} current (I_{Ca}) and $\text{Na}^+/\text{Ca}^{2+}$ exchanger current ($I_{\text{Na/Caex}}$) are candidate inward currents for initiating arrhythmia-triggering afterdepolarizations in electrically remodeled myocardium.^{10,11} Whereas CaMKII is hypothesized to favor afterdepolarizations attributable to I_{Ca} ⁵ and $I_{\text{Na/Caex}}$ ⁶ in cellular models, CaMKII has not been demonstrated to be a proarrhythmic signal in vivo.

We developed a mouse model of cardiac hypertrophy using transgenic (TG) expression of a constitutively active form of

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CaMKIV, where endogenous CaMKII activity can be readily distinguished from TG CaMKIV activity using specific inhibitors and substrates. These mice have cardiac hypertrophy and reduced systolic function² and significantly increased CaMKII activity and expression. We studied these mice to test the hypothesis that CaMKII can be a proarrhythmic signaling molecule in vivo. In this study, we report in vivo, cellular, and biochemical evidence that CaMKII is a critical signal for arrhythmias in cardiac hypertrophy.

Methods

CaMKIV Transgenic Mice

TG mice expressed a truncated, constitutively active form of human CaMKIV lacking the calmodulin-binding domain.² TG mice develop moderate cardiac hypertrophy and $\approx 50\%$ reduction in left ventricular ejection fraction. Experiments were performed on 8- to 24-week-old TG mice and wild-type (WT) littermate controls.

Electrocardiographic Telemetry

Mice were anesthetized (pentobarbital 33 $\mu\text{g/g}$ and ketamine 33 $\mu\text{g/g}$ IP) before placing a transmitter (Data Sciences International) into the abdominal cavity with subcutaneous electrodes in a lead I configuration. ECG intervals were determined in ambulatory, unanesthetized mice by signal averaging 10-second epochs every 5 minutes for a 30-minute baseline period. Continuous recording was performed for 30-minute intervals during arrhythmia screening. Signal-averaging was performed using custom software developed at Vanderbilt University. Interval measurements were performed without explicit knowledge of genotype, but QT interval prolongation was always apparent in the TG mice. QT intervals were corrected (QTc) for heart rate by a formula developed for mice.¹²

Arrhythmia Induction and Screening

Arrhythmias were categorized into 5 groups and assigned the following point values: no arrhythmias, 0 points; premature atrial or ventricular beats, 1 point; supraventricular tachycardia or paired premature ventricular beats, 2 points; bigeminal or trigeminal premature ventricular beats or nonsustained ventricular tachycardia (≥ 3 consecutive premature ventricular beats), 3 points; and sustained ventricular tachycardia (>10 consecutive premature ventricular beats) or polymorphic ventricular tachycardia, 4 points.

Mice were treated with the CaMK inhibitory agent KN-93 (10 to 30 $\mu\text{mol/kg}$ IP) or the inactive congener KN-92 (30 $\mu\text{mol/kg}$ IP)¹³ 10 minutes before isoproterenol (100 μg IP). KN-93 and KN-92 were tested in the same mouse on different days. ECGs were obtained from unanesthetized and unrestrained mice 30 minutes after isoproterenol. Recordings were analyzed offline and coded for arrhythmias.

CaMKII Activity and Expression

CaMKII activity was determined in the presence of Ca^{2+} /CaM from fresh ventricular homogenates^{5,14} using syntide 2, a synthetic substrate with ≈ 50 -fold selectivity for CaMKII over CaMKIV.¹⁵ Ventricular extracts (30 μg protein per lane) were analyzed by SDS-PAGE and immunoblotted using a CaMKII antibody that specifically recognizes the δ isoform (a generous gift from Dr H. Singer, SUNY, Albany, NY) and a control PP1 β -specific antibody.¹⁶ Blots were developed using colorimetric reagents with alkaline phosphatase conjugated secondary antibodies and digitized images were quantified using NIM Image.

Inhibitory Peptides

The CaMKII inhibitory peptide AC3-I (KKALHRQEAVDCL, $\text{IC}_{50} \approx 3 \mu\text{mol/L}$)¹⁷ (Macromolecular Resources) is a modified CaMKII substrate; AC3-C (KKALHAQERVDCL) is an inactive control peptide ($\text{IC}_{50} > 500 \mu\text{mol/L}$). AC3-I (40 to 100 $\mu\text{mol/L}$) and AC3-C (100 $\mu\text{mol/L}$) were dialyzed into cells for 5 to 10 minutes before

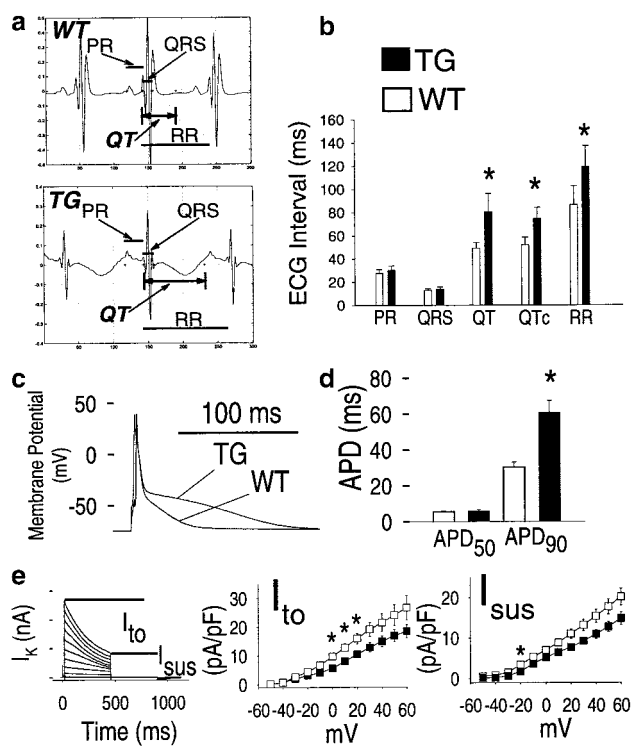


Figure 1. Electrical remodeling in TG mice. a, Signal-averaged ECGs show QT interval prolongation and abnormal QT interval displacement from the baseline in TG (bottom) compared with WT (top) mice. b, Summary ECG interval data from TG (n=8) and WT (n=6) mice. * $P < 0.005$ for TG compared with WT mice for all ECG intervals. c, Superimposed action potential recordings show repolarization is prolonged in TG cardiomyocytes. d, Summary data for APD₅₀ and APD₉₀ repolarization in TG (n=10) compared with WT (n=17) cardiomyocytes. * $P < 0.001$ for TG compared with WT. e, Transient (I_{to} , middle) and sustained (I_{sus} , right) components of repolarizing K^+ current are both reduced in TG (n=6) compared with WT (n=6) cardiomyocytes. Horizontal lines demarcate I_{to} and I_{sus} in this family of K^+ currents (left). * $P < 0.05$ for I_{to} and I_{sus} .

experiments. CaMKII inhibitory peptides were engineered for cell membrane permeability with separate minigenes encoding AC3-I and AC3-C using pGEX-3X-MTS2 (a generous gift from Drs Rojas and Lin, Vanderbilt University, Nashville, Tenn), as described.¹⁸ Cardiomyocytes were exposed to cell membrane permeant peptides (1 $\mu\text{mol/L}$) for ≥ 30 minutes before the experiments.

The $I_{\text{Na/CaX}}$ inhibitory peptide (XIP, RREIFYKYVYKRYRAGKQRF)¹⁹ and the inactive control peptide scrambled XIP (sXIP) (Macromolecular Resources) were dialyzed for 5 to 10 minutes (10 $\mu\text{mol/L}$) before initiating experiments.

Myocyte Isolation and Electrophysiology

Ventricular myocytes were isolated as previously described.²⁰

Voltage Clamp

Whole-cell mode voltage clamp measured transient (I_{to}) and sustained (I_{sus}) components of repolarizing K^+ current ($T = 34^\circ\text{C}$ to 36°C). I_{sus} was the residual current at the end of a 450-ms depolarizing pulse (0.33 Hz), and I_{to} was the difference between peak outward K^+ current and I_{sus} (Figure 1e).²¹ The bath solution for K^+ current voltage clamp studies was (in mmol/L) *N*-methyl-D-glucamine 149, HEPES 5, glucose 5, KCl 1, and MgCl_2 5, and the pH was adjusted to 7.4 with 12 N HCl.¹⁴ The pipette solution was (in mmol/L) K aspartate 120, HEPES 5, KCl 25, Na_2ATP 4, MgCl_2 1, Na_2 phosphocreatine 2, NaGTP 2, CaCl_2 1, and EGTA 10, and the pH was adjusted to 7.2 with 1 N KOH. On-cell mode voltage clamp

configuration was used to measure single LTCC currents, using Ba²⁺ or Ca²⁺ (both 110 mmol/L) as charge carrier, as previously described by us.²⁰ β -Adrenergic signaling was activated for LTCC recordings using isoproterenol (2 μ mol/L) and isobutylmethylxanthine (20 μ mol/L).²² Current clamp was used for stimulating action potentials (0.5 Hz) in physiological solutions (T=34°C to 36°C). Action potential duration was measured at 50% (APD₅₀) and 90% (APD₉₀) repolarization to baseline. The bath solution contained (in mmol/L) NaCl 140, HEPES 5, glucose 10, KCl 5.4, CaCl₂ 2.5, and MgCl₂ 1, and the pH was adjusted to 7.4 with 10 N NaOH. The pipette (intracellular) solution was the same as listed above for K⁺ current experiments. Junction potentials between pipette and bath solutions were compensated electronically. Early afterdepolarizations (EADs) were defined as discrete oscillations in repolarization during the action potential plateau.¹⁴

Statistics

The null hypothesis was rejected for $P < 0.05$ using Student's unpaired *t* test or ANOVA, as appropriate. The Wilcoxon ranked sign test was used for comparison of KN-93 and KN-92 effects on arrhythmias scores, and Fisher's exact test was used to compare the frequency of EADs between WT and TG mice. Data were expressed as mean \pm SEM.

Results

Electrical Remodeling in CaMKIV TG Mice

CaMKIV TG mice have increased QT intervals (Figures 1a and 1b), prolonged action potential durations (APDs) (Figures 1c and 1d), and reduced repolarizing K⁺ currents (Figure

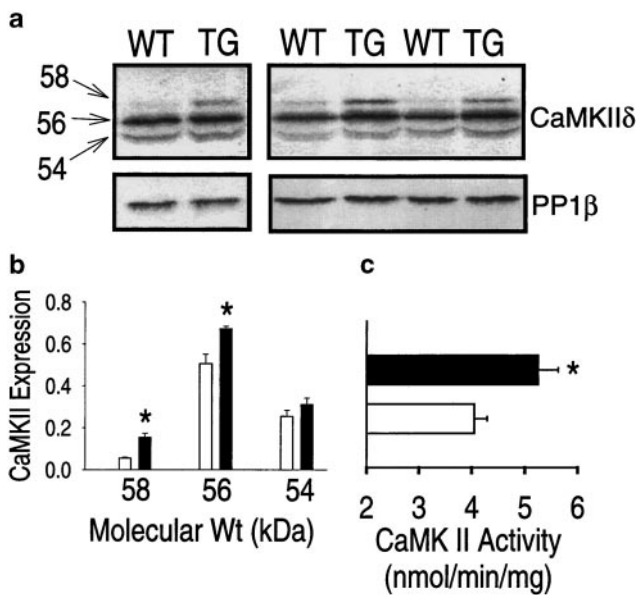


Figure 2. Increased cardiac CaMKII activity and expression in TG mice. a, Western blot studies of protein extracts (30 μ g/lane) from WT and TG hearts probed with a δ isoform-specific CaMKII antibody. The lanes show 3 separate pairs of heart extracts prepared in parallel from WT (n=5) or TG (n=5) mice. The CaMKII δ antibody detects a triplet of bands corresponding to 54, 56, and 58 kDa, consistent with reported different δ splice variants in human cardiomyopathy.⁷ In contrast, the catalytic subunit of the β isoform of protein phosphatase 1 (PP1 β) is unchanged in TG compared with WT and was used to normalize CaMKII expression. b, Densitometric analysis shows that δ CaMKII bands, migrating at 58 ($*P=0.002$) and 56 kDa ($*P=0.014$), are significantly increased in TG compared with WT. c, Total cardiac CaMKII activity is significantly increased ($*P < 0.05$) in TG mice (n=4) compared with WT mice (n=4).

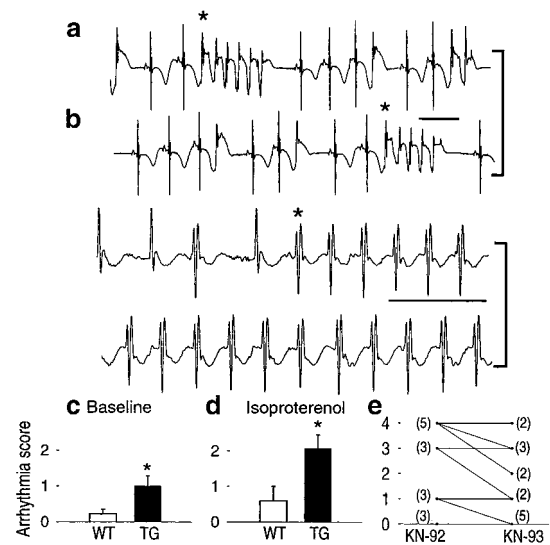


Figure 3. Cardiac arrhythmias recorded in telemetered, unrestrained, and unanesthetized TG mice are suppressed by CaMKII inhibition. a, Spontaneous polymorphic ventricular arrhythmia occurred in short bursts (*arrhythmia onset). b, Isoproterenol-induced ventricular tachycardia initiates abruptly (*arrhythmia onset) and is characterized by dissociation of atrial and ventricular complexes (seen as larger triphasic deflections). Scale bars indicate 200 ms for panels a and b. Arrhythmia scores (see Methods) were significantly higher at baseline in the TG (n=23) compared with WT (n=18) mice ($*P < 0.05$). d, Isoproterenol significantly increased the arrhythmia score in TG (n=14) compared with WT (n=5) mice ($*P < 0.05$). e, Systemic CaMKII inhibition with KN-93 significantly ($P=0.028$) suppressed isoproterenol-induced arrhythmias in TG mice compared with the inactive control agent, KN-92. Lines connect individual TG mice (n=14) treated with KN-92/KN-93 on different days, and bracketed numerals indicate total number of mice at each arrhythmia score. Data in panels d and e are from the same TG mice.

1e). These findings show that the CaMKIV TG mouse has important electrical changes seen generally in cardiomyopathy and suggest that it may be a useful new model to probe the molecular signaling mechanisms for arrhythmias in cardiac hypertrophy.

CaMKII Expression and Activity Are Increased in TG Hearts

CaMKII activity and expression are both increased in CaMKIV TG mice (Figure 2), as occurs in humans and other animal models of cardiomyopathy.⁷⁻⁹ Based on the combined presence of electrical remodeling (Figure 1) and increased CaMKII activity (Figure 2), we tested whether CaMKIV TG mice had arrhythmias and if these arrhythmias could be suppressed by CaMKII inhibition.

Increased Arrhythmias in TG Mice Are Suppressed by Inhibition of CaMK

Although one CaMKIV TG mouse had spontaneous torsade de pointes (Figure 3a), a form of ventricular tachycardia linked to Ca²⁺/calmodulin (CaM)-dependent signaling,^{23,24} and arrhythmias were significantly more common in TG than WT mice (Figure 3c), arrhythmia scores were low under basal conditions. Isoproterenol was administered to increase arrhythmia scores, based on the reasoning that β -adrenergic

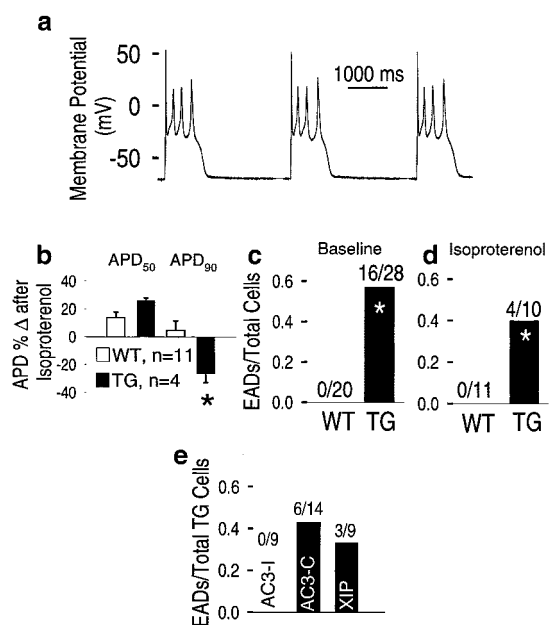


Figure 4. CaMKII activity is required for EADs. a, EADs are oscillations in the prolonged plateau phase of stimulated action potentials from TG mice. b, Isoproterenol increased APD₅₀ in TG (n=4) and WT (n=11) cardiomyocytes but significantly shortened APD₉₀ only in TG cells (**P*<0.05). c and d, EADs were frequent in TG cardiomyocytes at baseline (c, **P*<0.001) and after isoproterenol (d, **P*<0.05) but were never seen in WT littermate controls. The number of cells with EADs (numerator) and the total number of cells studied (denominator) is shown as a fraction. e, EADs in TG cardiomyocytes were prevented by dialysis of the CaMKII inhibitory peptide AC3-I but not by the inactive control peptide AC3-C (**P*=0.002), whereas the Na⁺/Ca²⁺ exchanger inhibitory peptide XIP failed to suppress EADs.

agonists are known to increase [Ca²⁺]_i,^{25–27} activate CaMKII,²⁶ and favor arrhythmia-triggering afterdepolarizations.^{25,27} Arrhythmias were observed significantly more frequently in unanesthetized and unrestrained TG mice at baseline, and high-grade arrhythmias (point score ≥2) occurred frequently in TG mice after isoproterenol (Figure 3), consistent with the increased tendency for arrhythmias in electrically remodeled myocardium.

To test the hypothesis that the enhanced CaMKII activity (Figure 2) contributed to high-grade arrhythmias that were frequent in TG mice after isoproterenol, TG mice were pretreated with the CaMK inhibitory agent KN-93 or the inactive congener KN-92. KN-93 selectively targets endogenous CaMK, because it acts as a competitive and noncompetitive inhibitor for CaM binding,¹³ and the CaM binding domain is absent on the TG CaMKIV.² These paired experiments showed KN-93 significantly reduced arrhythmia severity in TG mice (Figure 3e) but not in isoproterenol-treated WT mice (not shown), supporting the concept that CaMKII is a proarrhythmic molecule in this model of cardiac hypertrophy.

CaMKII Activity Is Required for EADs in CaMKIV TG Mice

EADs are an important trigger for arrhythmias in electrically remodeled myocardium,^{10,11} and EADs were only observed in

ventricular myocytes from TG mice at baseline (Figures 4a and 4c) and after isoproterenol (Figure 4d). Interestingly, EAD frequency was not affected by isoproterenol (*P*=0.46 compared with baseline for TG cells). Isoproterenol induced complex effects on APD, lengthening APD₅₀ in TG and WT cells but shortening APD₉₀ only in TG cardiomyocytes (Figure 4b). The findings up to this point show that TG mice have increased arrhythmias and frequent EADs and suggest that isoproterenol may enable EADs to more effectively trigger arrhythmias without increasing overall EAD frequency.

To examine the hypothesized cellular basis for the link between increased CaMKII activity and arrhythmias, we measured the response of EADs to the CaMKII inhibitory peptide AC3-I.¹⁷ CaMKII activity was required for EADs because EADs were prevented by AC3-I but not by the inactive control peptide AC3-C (Figure 4e). Both LTCC²⁰ and the Na⁺/Ca²⁺ exchanger⁶ cause inward currents regulated by CaMKII activity that may initiate EADs. However, a *I*_{Na/Caex} inhibitory peptide did not prevent EADs (Figure 4e), indicating that *I*_{Na/Caex} did not cause EADs in this model. EAD initiation was within the *I*_{Ca} window potential range (−27.5±0.2 mV for 534 EADs from 9 cells), suggesting that LTCC activity could be responsible for EADs seen in these cells.²⁸

Action Potential Prolongation Alone Does Not Cause EADs

EADs can be prevented by shortening action potential repolarization,²⁹ so we measured APD in isolated TG myocytes to

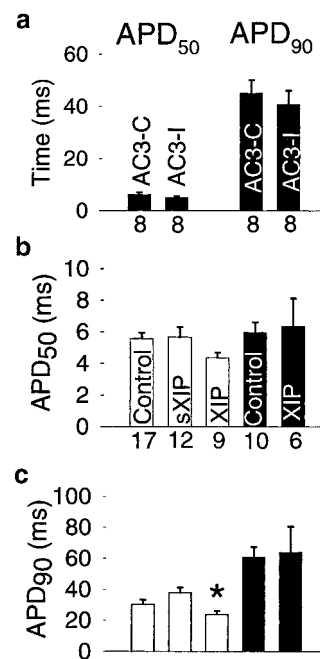


Figure 5. CaMKII inhibition does not shorten the APD in TG cardiomyocytes. a, APD₅₀ and APD₉₀ were not different between TG cardiomyocytes (black bars) dialyzed with the CaMKII inhibitory peptide AC3-I or the inactive control peptide AC3-C. b and c, The Na⁺/Ca²⁺ exchanger current inhibitory peptide (XIP) significantly shortened APD₉₀ (c, **P*=0.01) in WT cardiomyocytes (open bars) compared with control (no peptide) or an inactive scrambled XIP congener (sXIP). XIP did not shorten APD in TG cells. Labeled bars and the number of cells studied (abscissa) in panel b correspond to bars in panel c.

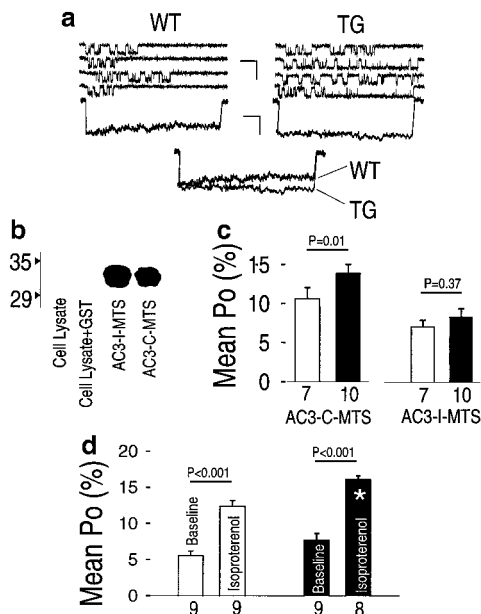


Figure 6. LTCC Po is increased in TG cardiomyocytes because of increased CaMKII activity. **a**, Single LTCC recordings (Ba^{2+} as charge carrier) in response to depolarizing voltage command pulses from WT (left) and TG (right) cardiomyocytes are shown in the top 4 tracings. Ensemble-averaged tracings (300 sweeps) are shown below the raw data tracings and superimposed to enhance comparison; similar results were found with Ca^{2+} as charge carrier (not shown). **b**, GST-linked, cell membrane permeant AC3-I-MTS and inactive control AC3-C-MTS peptides (2 $\mu\text{mol/L}$) effectively traversed cell membranes as seen from cell lysates showing prominent bands at ≈ 32 kDa in response to anti-GST antibodies. Untreated cell lysate or cells lysed after incubation with GST alone did not show evidence of GST immunoreactivity. **c**, LTCC Po was higher in TG (black bars) than WT cells (open bars) with Ba^{2+} (data shown here) or Ca^{2+} as charge carrier (**d**), and AC3-I-MTS eliminated this difference (right). **d**, LTCC Po is significantly increased in TG and WT cardiomyocytes by isoproterenol (2 $\mu\text{mol/L}$, Ca^{2+} as charge carrier), but LTCC Po remained significantly greater in TG compared with WT after isoproterenol ($*P=0.001$). Numerals indicate the number of cells studied.

determine if the mechanism of CaMKII inhibition in suppressing EADs was related to shortening repolarization. CaMKII inhibition did not shorten APD (Figure 5a). In contrast, the $I_{\text{Na}/\text{Ca}_{\text{ex}}}$ inhibitory peptide XIP shortened APD in WT but not TG ventricular myocytes (Figure 5c), indicating that XIP produced a significant effect at the concentration used in these experiments and suggesting that $I_{\text{Na}/\text{Ca}_{\text{ex}}}$ was not a critical determinant of the action potential prolongation seen in the electrically remodeled TG cardiomyocytes. Taken together, these findings indicate that CaMKII activation, and not APD prolongation alone, is required for EADs and that these EADs are attributable to a $I_{\text{Na}/\text{Ca}_{\text{ex}}}$ -independent conductance.

Increased LTCC Activity in TG Mice Is CaMKII Dependent

LTCC activity is increased in myopathic human hearts,³⁰ and CaMKII increases LTCC opening probability (Po) in normal adult cardiomyocytes,²⁰ suggesting the possibility that increased CaMKII activity in TG mouse hearts (Figure 2) and

diseased human hearts⁷ could drive increased LTCC activity. LTCC Po was higher in TG than WT cells at baseline (Figures 6a and 6c) and after isoproterenol (Figure 6d). To determine if increased CaMKII activity (Figure 2) was the molecular mechanism for increased LTCC Po in TG mice, we developed a cell membrane permeant form of AC3-I (Figure 6b). AC3-I, but not a cell membrane permeant control peptide, eliminated the increased LTCC Po present in TG mice (Figure 6c). These findings indicate that CaMKII activity is the cause of increased LTCC activity seen in the TG mice at baseline and suggest a mechanistic framework for understanding the efficacy of acute suppression of CaMKII in reducing arrhythmias (Figure 3) and EADs (Figure 4).

Discussion

CaMK and the Arrhythmogenic Phenotype in Cardiomyopathy

CaMK types I,² II,^{3,33} and IV² are all implicated in cardiac hypertrophy, but CaMKII is by far the most abundant CaM-activated kinase in heart. Upregulation of CaMKII seems to be a general feature of cardiomyopathy in humans,⁷ rats,⁸ and mice (Figure 2), although the mechanism for CaMKII upregulation is presently unknown. CaMKII targets key control molecules for intracellular Ca^{2+} homeostasis in cardiomyocytes, and increased CaMKII may trigger arrhythmia-initiating afterdepolarizations⁵ by activating LTCCs²⁰ or increasing $I_{\text{Na}/\text{Ca}_{\text{ex}}}$ during action potential prolongation.⁶ The present results support the hypothesis that CaMKII is a critical molecular signal for arrhythmias in cardiac hypertrophy in vivo and suggest that increased LTCC Po and EADs may be CaMKII-driven components of the cellular arrhythmia mechanism.

Isoproterenol-Induced Arrhythmias

The present findings support the concept that the increased CaMKII present in cardiomyopathy is arrhythmogenic and that CaMKII-dependent arrhythmias are additionally enhanced after activation through β -adrenergic stimulation, perhaps by virtue of the increased $[\text{Ca}^{2+}]_i$ that follows generation of protein kinase A.²⁶ On the other hand, β -adrenergic receptor blockade significantly reduces sudden death in patients with cardiac hypertrophy and heart failure,³¹ raising the possibility that part of the salutary effect of β -adrenergic receptor antagonist drugs may be mediated through secondary actions on CaMKII signaling.

Arrhythmia Mechanisms in Cardiomyopathy

There is an increasing recognition that arrhythmia mechanisms in cardiomyopathy involve both cellular and tissue remodeling. Cellular studies consistently reveal APD prolongation and an increased tendency for afterdepolarizations that are a hypothesized focal mechanism for arrhythmia triggering.^{10,11} Focal cellular mechanisms seem to be important for arrhythmia initiation in patients with cardiomyopathy,³² but changes in the myocardium, involving intercellular coupling, scarring, and fibrosis, also constitute a macroscopic arrhythmogenic substrate that contributes to arrhythmia maintenance. The TG mice have more arrhythmias and frequent EADs at baseline, but the finding that EAD frequency is

unchanged whereas arrhythmias increase after isoproterenol suggests that the proarrhythmic action of isoproterenol occurs at the tissue level, perhaps by enhancing EAD propagation. The present studies are the first to implicate CaMKII-dependent signaling in arrhythmias in cardiac hypertrophy. Additional studies will be required to address the effects of CaMKII on macroscopic arrhythmia mechanisms.

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