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Restoration of normal L-type Ca^{2+} channel function during Timothy syndrome by ablation of an anchoring protein

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Abstract

Rationale—L-type Ca^{2+} ($Ca_V1.2$) channels shape the cardiac action potential waveform and are essential for excitation-contraction coupling in heart. A gain-of-function G406R mutation in a cytoplasmic loop of $Ca_V1.2$ channels causes long QT syndrome 8 (LQT8), a disease also known as Timothy syndrome. However, the mechanisms by which this mutation enhances $Ca_V1.2$-LQT8 currents and generates lethal arrhythmias are unclear.

Objective—To test the hypothesis that the anchoring protein AKAP150 modulates $Ca_V1.2$-LQT8 channel gating in ventricular myocytes.

Methods and Results—Using a combination of molecular, imaging, and electrophysiological approaches, we discovered that $Ca_V1.2$-LQT8 channels are abnormally coupled to AKAP150. A pathophysiological consequence of forming this aberrant ion channel-anchoring protein complex is enhanced $Ca_V1.2$-LQT8 currents. This occurs through a mechanism whereby the anchoring protein functions like a subunit of $Ca_V1.2$-LQT8 channels that stabilizes the open conformation and augments the probability of coordinated openings of these channels. Ablation of AKAP150 restores normal gating in $Ca_V1.2$-LQT8 channels and protects the heart from arrhythmias.

Conclusion—We propose that AKAP150-dependent changes in $Ca_V1.2$-LQT8 channel gating may constitute a novel general mechanism for $Ca_V1.2$-driven arrhythmias.

Keywords

$Ca_V1.2$ channels; EC coupling; calcium; arrhythmias

Introduction

L-type Ca^{2+} ($Ca_V1.2$) channels are expressed in the sarcolemma of atrial and ventricular myocytes where they play a critical role in activating Ca^{2+} release from the sarcoplasmic reticulum (SR) during excitation-contraction (EC) coupling. The magnitude and time course of the $Ca_V1.2$ current determines the waveform of the cardiac action potential (AP)[1]. Thus,

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Disclosures
None.

changes in $Ca_V1.2$ channel function can have profound effects on cardiac EC coupling and excitability. Accordingly, a recent study[2] discovered that a single amino acid substitution (G406R) in $Ca_V1.2$ is linked to Timothy syndrome. Timothy syndrome is characterized by prolongation of the electrocardiogram (ECG) QT interval and lethal arrhythmias, which is why it is also known as long QT syndrome 8 (LQT8). Interest in the mechanisms of LQT8 has been intense because it is a multisystem disease, with many patients also afflicted by autism. Thus, a single amino acid mutation in $Ca_V1.2$ causes clinically significant disorders in the cardiac and central nervous systems.

Electrophysiological studies have revealed two distinctive features of LQT8 mutant $Ca_V1.2$ channels ($Ca_V1.2$-LQT8). *First*, these channels inactivate at a slower rate than wild type (WT) channels[2-4]. *Second*, small clusters of $Ca_V1.2$-LQT8 channels have a higher probability of undergoing coordinated openings and closings ("coupled gating") than WT channels[5]. Although recent reports suggest that the G406R substitution in $Ca_V1.2$ creates a new phosphorylation site for the Ca^{2+}/calmodulin-dependent kinase II (CaMKII), which contributes to an increase in the open probability (P_o) of $Ca_V1.2$-LQT8 channels, others suggest that phosphorylation by CaMKII is *not* necessary for their slower rate of inactivation[4, 6, 7]. Thus, the mechanism by which the activity of $Ca_V1.2$-LQT8 channels is coordinated to generate irregular cardiac rhythm is unclear.

A potential mechanism regulating the activity of $Ca_V1.2$-LQT8 channels involves the anchoring protein AKAP150. AKAP150 targets specific protein kinases and phosphatases to regions near $Ca_V1.2$ channels in ventricular myocytes and neurons[8, 9] Furthermore, AKAP150 binds to the carboxyl tail of $Ca_V1.2$ channels via leucine zipper (LZ) motifs in these two proteins[10], facilitating physical interactions between $Ca_V1.2$ carboxyl tails. AKAP150 increases the probability of long openings and coupled gating events between $Ca_V1.2$ channels[5]. At present, however, whether the interaction with AKAP150 modulates the abnormal $Ca_V1.2$-LQT8 channel activity is unknown.

Here, we employed a combination of cellular, molecular, imaging, and electrophysiological approaches to investigate this important issue. We discovered that AKAP150 is required for abnormal gating of $Ca_V1.2$-LQT8 channels. Importantly, our data indicate that ablation of AKAP150 corrects arrhythmogenic $Ca_V1.2$-LQT8 channel activity in ventricular myocytes.

Methods

An expanded Methods section is available in the Online Supplemental Material at http://circres.ahajournals.org.

Mice were euthanized using a lethal dose of sodium pentobarbital as approved by the University of Washington Institutional Animal Care and Use Committee. Details about the generation of our LQT8 mouse are available in the Online Supplemental Material. Ventricular myocytes were isolated as described previously[8]. Electrophysiological signals were recorded using HEKA EPC10 or Axopatch 200B amplifiers. Images were obtained using a confocal microscope. Data are presented as mean ± SEM. A p value of less than 0.05 was considered significant. An asterisk (*) was used in the figures to illustrate a significant difference between groups.

Results

Ablation of AKAP150 protects against cardiac hypertrophy during LQT8

We generated a transgenic mouse that expresses $Ca_V1.2$-LQT8 channels fused to the tag-red fluorescent protein (tRFP) solely in cardiac myocytes (LQT8; Figure 1A) and crossed them

with AKAP150 null mice (LQT8/AKAP150$^{-/-}$)[11]. Online Table I summarizes 21 different anatomical and functional features of these mice. We found that the heart-to-body weight ratio of LQT8 hearts was larger than that of WT, AKAP150$^{-/-}$, and LQT8/AKAP150$^{-/-}$ mice. Indeed, LQT8 myocytes were longer and wider than WT, AKAP150$^{-/-}$, and LQT8/AKAP150$^{-/-}$ myocytes. These findings suggest that expression of $Ca_V1.2$-LQT8 promotes cardiac hypertrophy and loss of AKAP150 protects LQT8 mice against it.

AKAP150 is not required for the expression or spatial organization of $Ca_V1.2$-LQT8 channels in adult ventricular myocytes

Western blot analysis of biotinylated endogenous WT $Ca_V1.2$ ($Ca_V1.2$-WT) and $Ca_V1.2$-LQT8 indicated that sarcolemmal $Ca_V1.2$-WT expression was similar in WT, LQT8, AKAP150$^{-/-}$, and LQT8/AKAP150$^{-/-}$ myocytes (Figure 1B). $Ca_V1.2$-LQT8 channels comprised 41 ± 5 (n = 6 mice) and 43 ± 4% (n = 6 mice) of the total sarcolemmal $Ca_V1.2$ population in LQT8 and LQT8/AKAP150$^{-/-}$ myocytes, respectively. Like $Ca_V1.2$-WT channels in WT and AKAP150$^{-/-}$ myocytes, $Ca_V1.2$-LQT8 channels were prominently expressed along the transverse tubules (T-tubules) of LQT8 and LQT8/AKAP150$^{-/-}$ myocytes. However, unlike $Ca_V1.2$-WT channels, $Ca_V1.2$-LQT8 channels were also expressed in the intercalated discs and seemed to form multiple clusters in the sarcolemma and near the nuclear envelope of LQT8 and LQT8/AKAP150$^{-/-}$ cells (Figure 1C). The number of $Ca_V1.2$-LQT8 clusters were similar in LQT8 (Online Figure II, 154 ± 7 clusters/cell, n = 7) and LQT8/AKAP150$^{-/-}$ cells (142 ± 68 clusters/cell, n = 5; p >0.05) (see Online Supplemental material for a description of this analysis). Collectively, these data suggest that $Ca_V1.2$-LQT8 and CaV1.2-WT channels are differentially expressed in ventricular myocytes, but that AKAP150 does not regulate the expression or distribution of these channels in these myocytes.

Loss of AKAP150 restores normal inactivation of I_{Ca} in LQT8 myocytes

We recorded macroscopic $Ca_V1.2$ currents (I_{Ca}) from WT, AKAP150$^{-/-}$, LQT8, and LQT8/AKAP150$^{-/-}$ ventricular myocytes. Although the amplitude of I_{Ca} was similar in WT, AKAP150$^{-/-}$, LQT8, and LQT8/AKAP150$^{-/-}$ ventricular myocytes (p > 0.05), there were striking differences in the rate of inactivation of these currents (Figure 2A-B and Online Table I). Indeed, the fraction of I_{Ca} remaining 50 ms (r_{50}) after the onset of depolarization to +10 mV from LQT8 myocytes was larger (n = 8) than in WT (n = 9) and AKAP150$^{-/-}$ myocytes (n = 5; p < 0.05), suggesting expression of functional $Ca_V1.2$-LQT8 channels in LQT8 myocytes. Indeed, from these I_{Ca} currents, we determined that $Ca_V1.2$-LQT8 channels account for ≈32% of the total $Ca_V1.2$ channel population in LQT8 myocytes (see Supplemental Material). Interestingly, the r_{50} of I_{Ca} in LQT8/AKAP150$^{-/-}$ (n = 9) was similar to that of WT and AKAP150$^{-/-}$. These data suggest that loss of AKAP150 restores normal I_{Ca} inactivation in LQT8 myocytes.

Our I_{Ca} data raise an important question: is AKAP150 required for the expression of functional $Ca_V1.2$-LQT8 channels? To address this question, we expressed these channels in WT and AKAP150$^{-/-}$ mouse embryonic fibroblasts (MEFs). As shown in Figure 2C, we recorded robust I_{Ca} (1-3 pA/pF) only in cells transfected with $Ca_V1.2$-WT or $Ca_V1.2$-LQT8. In WT MEFs (Figure 2C-D and Online Table I), $Ca_V1.2$-LQT8 currents (r_{50} = 0.73 ± 0.10, n = 5) inactivated at a much slower rate than $Ca_V1.2$-WT currents at +10 mV (r_{50} = 0.25 ± 0.02, n = 6; p < 0.05). However, in AKAP150$^{-/-}$ MEFs, $Ca_V1.2$-LQT8 channels (r_{50} = 0.28 ± 0.03, n = 5) produced currents with a similar time course to that of $Ca_V1.2$-WT channels (r_{50} = 0.35 ± 0.03 at +10 mV; n = 5 cells; p > 0.05). Thus, although AKAP150 is not necessary for the expression of functional WT or LQT8 $Ca_V1.2$ channels, it is required for defective inactivation of $Ca_V1.2$-LQT8 channels.

A potential mechanism by which AKAP150 could promote a slow rate of inactivation of $Ca_V1.2$-LQT8 currents is by acting as an anchor for protein kinase A (PKA)[8, 10]. Another possibility is that the effects of AKAP150 on $Ca_V1.2$-LQT8 channel inactivation depend on CaMKII activity. Application of ht31 (PKA-AKAP interaction inhibitor, 10 μM), Rp-cAMP (PKA inhibitor, 100 μM), or KN-93 (CaMKII inhibitor, 5 μM) did not change the r_{50} of I_{Ca} in LQT8 myocytes (Online Figure I, $p > 0.05$), which suggests that PKA or CaMKII activity is not responsible for the potentiation of I_{Ca} during LQT8. Furthermore, these data support the view that the necessity of AKAP150 for decreased $Ca_V1.2$-LQT8 channel inactivation is not dependent on CaMKII activity or its ability to target PKA locally.

AKAP150 is required for increased $Ca_V1.2$ channel activity and coupled gating seen in LQT8 myocytes

To test the hypothesis that ablation of AKAP150 decreases the P_o, open time, and frequency of coupled gating events by $Ca_V1.2$ channels in LQT8 myocytes, we recorded the *in situ* activity of $Ca_V1.2$ channels in WT, LQT8, and LQT8/AKAP150$^{-/-}$ myocytes using the cell-attached configuration of the patch clamp technique (Figure 3A and Online Table I). AKAP150$^{-/-}$ myocytes were not included in these experiments because the amplitude, rate of inactivation, and voltage-dependence of I_{Ca} in these cells is similar to that of WT cells and LQT8/AKAP150$^{-/-}$ cells. Thus, it is unlikely that single $Ca_V1.2$ channel activity in AKAP150 null myocytes would be different to that of WT and LQT8/AKAP150$^{-/-}$ cells.

The amplitudes of elementary Ca^{2+} currents were similar in WT (0.55 ± 0.10 pA, n = 8 cells), LQT8 (0.60 ± 0.11 pA, n = 12 cells), and LQT8/AKAP150$^{-/-}$ (0.58 ± 0.12 pA, n = 10 cells) myocytes at -30 mV ($p > 0.05$). Consistent with our I_{Ca} data, the activity (i.e., NP_o where N is the number of channels and P_o is the open probability) of $Ca_V1.2$ channels in LQT8 myocytes (0.11 ± 0.04) was ≈10-fold higher than in WT (0.01 ± 0.01) and LQT8/AKAP150$^{-/-}$ (0.02 ± 0.01) myocytes ($p < 0.05$; Figure 3B). Furthermore, analysis of the open dwell times from $Ca_V1.2$ channels revealed that a larger proportion of channel openings are long openings in LQT8 myocytes than those recorded from LQT8/AKAP150$^{-/-}$ and WT myocytes. The open time histograms from WT and LQT8/AKAP150$^{-/-}$ myocytes could be fit with a single exponential function with a time constant (τ_{short}) of 0.8 ms and 0.6 ms, while the open time histogram of $Ca_V1.2$ channels in LQT8 myocytes could be fit with the sum of two exponential functions with τ_{short} of 1.3 ms and τ_{long} of 9.4 ms, which accounted for 95% and 5% of the channel openings, respectively (Figure 3B). The time constants from LQT8 myocytes likely represents a mixed population of WT and LQT8 $Ca_V1.2$ channels operating in two gating modalities in LQT8 myocytes. By contrast, the long $Ca_V1.2$ channel openings observed in LQT8 myocytes were completely absent in LQT8/AKAP150$^{-/-}$ cells. Collectively, these data suggest that AKAP150 is required for long openings of $Ca_V1.2$ channels in LQT8 myocytes.

To test the hypothesis that $Ca_V1.2$-LQT8 channels have a higher probability of coupled gating than $Ca_V1.2$-WT channels in ventricular myocytes, we implemented a coupled Markov chain model to determine the coupling coefficient (κ) among Ca_V 1.2 channels[5, 12]. The mean coupling coefficient was 0.13 ± 0.03 for Ca^{2+} channels in LQT8 myocytes and 0.03 ± 0.01 for WT and 0.03 ± 0.01 for LQT8/AKAP$^{-/-}$ cells (Figure 3D). Indeed, the frequency of coupled gating events (κ > 0.1) was higher in LQT8 (43 ± 10%) myocytes than in WT (8 ± 4%) and LQT8/AKAP150$^{-/-}$(10 ± 6%) myocytes ($p < 0.05$; Figure 3E).

Loss of AKAP150 restores normal $[Ca^{2+}]_i$, AP waveform, and cardiac rhythm in LQT8 mice

We recorded AP-evoked $[Ca^{2+}]_i$ transients in WT, LQT8, AKAP150$^{-/-}$, and LQT8/AKAP150$^{-/-}$ myocytes (Figure 4A and Online Table I). The amplitudes of the AP-evoked $[Ca^{2+}]_i$ transient in WT myocytes (n = 7), AKAP150$^{-/-}$ (n = 7), and LQT8/AKAP150$^{-/-}$

myocytes (n = 9) were similar ($p > 0.05$). The $[Ca^{2+}]_i$ transient was larger in LQT8 myocytes (n = 9) than in these myocytes ($p < 0.05$). Furthermore, although 56% of LQT8 myocytes had spontaneous Ca^{2+} release (SCR) events under control conditions, none was detected in WT, AKAP150$^{-/-}$, or LQT8/AKAP150$^{-/-}$ myocytes under similar experimental conditions. Because AKAP150 is required for β-adrenergic induced increases in the amplitude of the AP-evoked $[Ca^{2+}]_i$ transient in ventricular myocytes[8], we examined the effects of the β-adrenergic agonist isoproterenol (ISO, 100 nM) on WT, LQT8, AKAP150$^{-/-}$, and LQT8/AKAP150$^{-/-}$ myocytes (Figure 4A and Online Table I). We found that ISO increased the amplitude of the AP-evoked $[Ca^{2+}]_i$ in WT and LQT8, but not in AKAP150$^{-/-}$ or LQT8/AKAP150$^{-/-}$ myocytes, providing functional confirmation of the loss of AKAP150 in these cells ($p < 0.05$). ISO also increased the number of spontaneous Ca^{2+} release events in $Ca_V1.2$-LQT8 cells from 40 to 85%, but not in WT, AKAP150$^{-/-}$, and LQT8/AKAP150$^{-/-}$ myocytes.

We investigated whether restoration of normal inactivation of I_{Ca} in LQT8/AKAP150$^{-/-}$ myocytes translated to changes in AP waveform in these cells. Consistent with our I_{Ca} data, the duration of the AP at 90% repolarization (APD_{90}) was longer in LQT8 (n = 10) than in WT (n = 5), AKAP150$^{-/-}$ (n = 5), and LQT8/AKAP150$^{-/-}$ (n = 11) myocytes ($p < 0.05$; Figure 4B and Online Table I). In addition, analysis of records with trains of APs revealed that LQT8 myocytes had a higher frequency of early (EADs) and delayed afterdepolarizations (DADs) than WT, AKAP150$^{-/-}$, and LQT8/AKAP150$^{-/-}$ myocytes (Figure 4C and Online Table I).

To determine the electrophysiological phenotype of WT, LQT8, AKAP150$^{-/-}$, and LQT8/AKAP150$^{-/-}$ mice, we implanted telemetric ECG transmitters[13] (Figure 4D and Online Table I). Heart rate was similar in WT (n = 6), LQT8 (n = 5), AKAP150$^{-/-}$ (n = 6), and LQT8/AKAP150$^{-/-}$ at rest (n = 6) or during mild exercise ($p > 0.05$). However, consistent with our I_{Ca} and AP data, the QT interval — corrected for heart rate using Bazet's formula (i.e., QT_c) — of LQT8 mice (116 ± 1 ms) is longer than that of WT (97 ± 1 ms), AKAP150$^{-/-}$ (98 ± 1), and LQT8/AKAP150$^{-/-}$ mice (108 ± 1 ms; $p < 0.05$). During exercise, although multiple premature ventricular depolarizations (PVDs) and epiodes of *torsades de pointes* (TdPs, a hallmark of LQT) were observed in LQT8 mice, none was recorded from WT, AKAP150$^{-/-}$, and LQT8/AKAP150$^{-/-}$ mice (Figure 4D and Online Table I). Thus, loss of AKAP150 was protective against arrhythmias in mice expressing $Ca_V1.2$-LQT8.

Discussion

Our findings suggest a new model of $Ca_V1.2$-LQT8 channel dysfunction during Timothy syndrome (Figure 4E). In this model, the anchoring protein AKAP150 and $Ca_V1.2$-LQT8 form a complex that is necessary for aberrant $Ca_V1.2$-LQT8 channel gating and arrhythmias. $Ca_V1.2$-LQT8 channels likely interact with AKAP150 via LZ motifs in the carboxyl tails of both proteins[10]. We propose that AKAP150 functions like an allosteric modulator of $Ca_V1.2$-LQT8 channels, increasing $Ca_V1.2$-LQT8 currents by stabilizing the open conformation and increasing the probability of coupled gating between $Ca_V1.2$-LQT8 channels. This leads to increased Ca^{2+} influx, AP prolongation, cardiac hypertrophy, and arrhythmias. Coupled gating of $Ca_V1.2$-LQT8 channels presumably occurs because AKAP150 promotes physical interactions of adjacent channels via their carboxyl tails[5, 10, 14].

Our data provide insights into the cellular mechanisms by which $Ca_V1.2$-LQT8 channels increase the probability of arrhythmias. We found that expression of $Ca_V1.2$-LQT8 channels increased the frequency of arrhythmogenic EADs and DADs. EADs are likely produced by reactivation of $Ca_V1.2$ channels during the long APs of LQT8 myocytes. It is intriguing to

speculate that the larger Ca^{2+} influx associated with $Ca_V1.2$-LQT8 channels leads to SR Ca^{2+} overload and thus to SRC events and DADs in LQT8 myocytes. Future experiments should examine in detail the relationship between Ca^{2+} influx via $Ca_V1.2$-LQT8 and EADs and DADs in these cells.

Ablation of AKAP150 corrects pathological $Ca_V1.2$-LQT8 channel gating and arrhythmias and prevents hypertrophy of LQT8 hearts presumably by decreasing Ca^{2+} influx via $Ca_V1.2$-LQT8 channels. Because AKAP150 does not bind CaMKII, loss of this scaffolding protein is not expected to affect CaMKII-dependent modulation of $Ca_V1.2$-LQT8 channels in ventricular myocytes. However, our data suggest that AKAP150 is required for any potential CaMKII-induced changes in $Ca_V1.2$-LQT8 gating. Thus, we propose that disrupting the interaction between AKAP150 and $Ca_V1.2$-LQT8 is a potential target for novel therapeutics for treating the broad spectrum of Timothy syndrome's symptoms, including lethal arrhythmias and autism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AKAP150	A-kinase anchoring protein 150
AP	action potential
$[Ca^{2+}]_i$	intracellular Ca^{2+} concentration
CaM	calmodulin
CaMKII	Ca^{2+}/calmodulin-dependent kinase II
$Ca_V1.2$-LQT8	$Ca_V1.2$ channels with the long QT syndrome mutation
$Ca_V1.2$-WT	wild type $Ca_V1.2$ channels
EC coupling	excitation-contraction coupling
ECG	electrocardiogram
LQT8	long QT syndrome 8 (Timothy syndrome)
LZ	leuzine zipper
MEF	mouse embryonic fibroblast
TdP	Torsades de pointes
tRFP	tag red fluorescent protein
WT	wild type

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Novelty and Significance

What is known?

- A single amino acid substitution in $Ca_V1.2$ L-type Ca^{2+} channels causes long QT syndrome 8 (LQT8).
- $Ca_V1.2$-LQT8 channels are characterized by an abnormally slow rate of inactivation and by exhibiting a high frequency of coordinated openings between nearby channels.
- The A-kinase anchoring protein 150 (AKAP150) is a $Ca_V1.2$ channel-associated scaffolding protein that regulates $Ca_V1.2$ channel function and excitation-contraction (EC) coupling by targeting adenyl cyclase 5, protein kinase A, and calcineurin near these channels.

What New Information Does This Article Contribute?

- AKAP150 is required for the expression of the LQT8 phenotype in a mouse model of this disease.
- AKAP150 functions like an allosteric modulator of $Ca_V1.2$-LQT8 channels that increases the opening time and also facilitates coupled gating between these channels in LQT8 cardiac myocytes.
- AKAP150 directly modulates the gating of $Ca_V1.2$-LQT8 without the aid of kinases.

The mechanism by which the LQT8 mutation alters the function of $Ca_V1.2$-LQT8 and EC coupling is unclear. Here, we establish that interaction between $Ca_V1.2$ and AKAP150 is necessary for the expression of the LQT8 phenotype. We find that AKAP150 functions as an accessory protein to the mutant $Ca_V1.2$-LQT8 channels, directly modulating the gating of these channels independently of its role in targeting adrenergic signaling. We also find that the coupled gating modality plays an important role in the pathophysiology of LQT8. The increased activity of $Ca_V1.2$-LQT8 in complex with AKAP150 increases the frequency of arrhythmogenic voltage fluctuations and arrhythmias. Our findings establish a novel role for AKAP150 as a $Ca_V1.2$ accessory protein in LQT8, and suggest that disruption of the interaction between $Ca_V1.2$ and AKAP150 could be a potential novel therapeutic target for LQT8 and other arrhythmias.

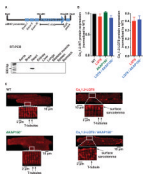

Figure 1. AKAP150 is not required for the expression and spatial distribution of $Ca_V1.2$-LQT8 channels in ventricular myocytes

(A) Cardiac-specific expression of $Ca_V1.2$-LQT8 channels was achieved by using the α-myosin heavy chain (αMHC) promoter. The lower panel shows that expression of $Ca_V1.2$-LQT8 transcript was cardiac specific in LQT8 mice. **(B)** Sarcolemmal WT and LQT8 $Ca_V1.2$ protein expression in WT, LQT8, AKAP150$^{-/-}$, and LQT8/AKAP150$^{-/-}$ myocytes. **C)** Confocal images of WT or LQT8 $Ca_V1.2$ channel-associated fluorescence in WT (immunofluorescence), LQT8 (tRFP fluorescence), AKAP150 (immunofluorescence), and LQT8/AKAP150$^{-/-}$ myocytes (tRFP fluorescence). Below each image, the section of the cell contained within the white rectangles is shown at higher magnification.

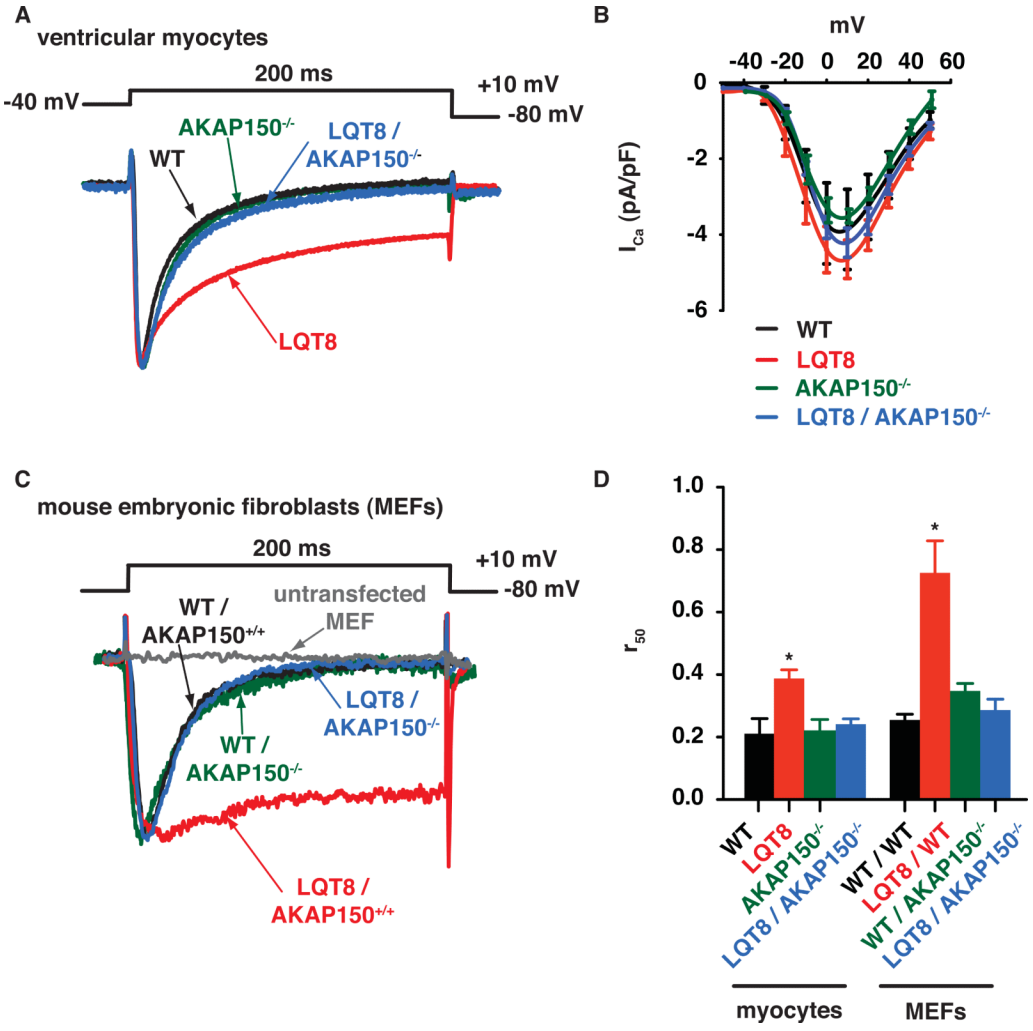

Figure 2. Loss of AKAP150 restores normal inactivation of I_{Ca} in LQT8 myocytes

(A) Normalized I_{Ca} records from representative WT, LQT8, AKAP150$^{-/-}$, and LQT8/AKAP150$^{-/-}$ ventricular myocytes. **(B)** Current-voltage relationship of I_{Ca} in WT, LQT8, AKAP150$^{-/-}$, and LQT8/AKAP150$^{-/-}$ myocytes. **(C)** I_{Ca} records from WT and AKAP150$^{-/-}$ MEFs expressing either WT or LQT8 $Ca_V1.2$ channels. A current record from an untransfected MEF is also shown. (**D)** Bar plot of the fraction r_{50} in ventricular myocytes or MEFs.

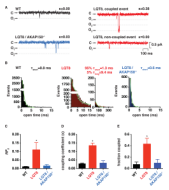

Figure 3. AKAP150 is required for increased in $Ca_V1.2$ channel activity and coupled gating seen in LQT8 myocytes

(A) Exemplar cell-attached $Ca_V1.2$ channel currents from membrane patches recorded during a step depolarization to -30 mV from -80 mV, with various coupling coefficients (κ) from WT, LQT8, and LQT8/AKAP150$^{-/-}$ ventricular myocytes. The 0 pA current level is marked by C. Dashed grey lines show the amplitude of opening for 1 (O_1), 2 (O_2), or 3 (O_3) channels. **(B)** Open dwell time histograms of $Ca_V1.2$ channel openings in WT (n = 8 cells, 1 patch/cell), LQT8 (n = 12 cells), and LQT8/AKAP150$^{-/-}$ (n = 10 cells) myocytes. The time constants (τ) of exponential function fits (green line) of these histograms are shown. In LQT8 patches, a two-term exponential fit with a τ_{short} and τ_{long} of 1.3 and 9.4 ms represent 95% and 5% of the entire population is optimal. Bar plots of the NP_O, κ, and the fraction of records with κ values > 0.05 are shown in panels **C, D**, and **E**, respectively.

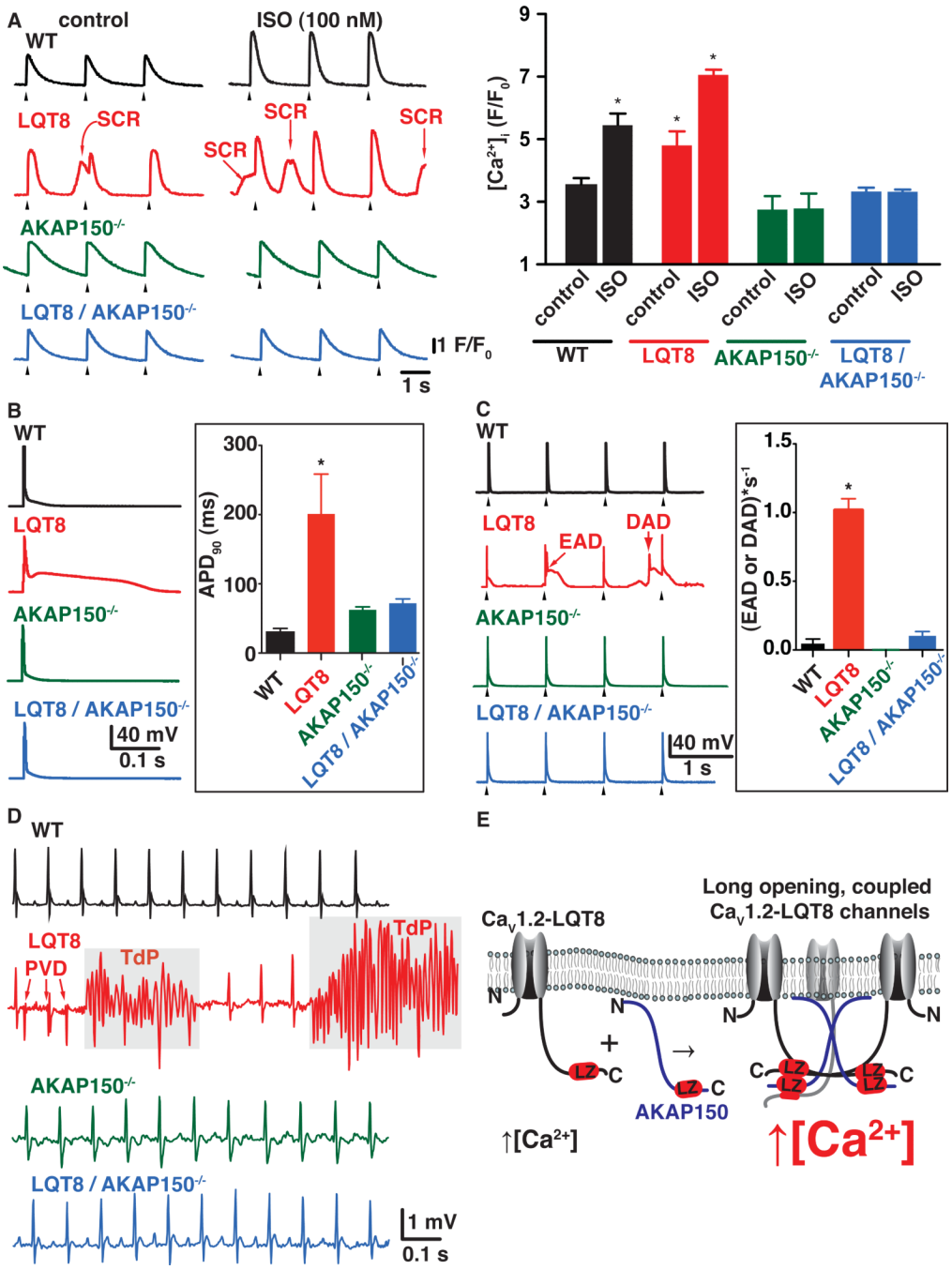

Figure 4. Loss of AKAP150 restores normal $[Ca^{2+}]_i$, AP waveform, and cardiac rhythm in LQT8 mice

(A) $[Ca^{2+}]_i$ transients from representative WT, LQT8, and LQT8/AKAP150$^{-/-}$ myocytes before and after the application of 100 nM ISO. Spontaneous Ca^{2+} release events (SCR) in LQT8 myocytes are indicated. Arrowheads below indicate external stimuli. Bar plot represents the $[Ca^{2+}]_i$ transient amplitudes. **(B)** APs from WT, LQT8, and LQT8/AKAP150$^{-/-}$ myocytes. (*Inset*) Bar plot of APD_{90}. **(C)** Trains of APs recorded from WT, LQT8, AKAP150$^{-/-}$ and LQT8/AKAP150$^{-/-}$ myocytes. EAD and DAD are indicated. Arrowheads below indicate current injection. The inset shows a bar plot of the rate of EADs or DADs in WT, LQT8, AKAP150$^{-/-}$, and LQT8/AKAP150$^{-/-}$ myocytes. **(D)** ECG traces

from WT, LQT8, and LQT8/AKAP150$^{-/-}$ mice. PVDs in the LQT8 trace are marked by arrows. The gray box highlights TdP in this LQT8 mouse. **(E)** Proposed model of how AKAP150 binds to the C-terminal tail of $Ca_V1.2$-LQT8 channels, facilitating longer channel openings and interaction between multiple $Ca_V1.2$-LQT8 channels, which increases the frequency of coupled gating and greater Ca^{2+} influx, leading to arrhythmias.