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CaMKII inhibition rescues pro-arrhythmic phenotypes in model of human ankyrin-B syndrome

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Abstract

Background—Cardiovascular disease is a leading cause of death worldwide. Arrhythmias are associated with significant morbidity and mortality related to cardiovascular disease. Recent work illustrates that many cardiac arrhythmias are initiated by a pathologic imbalance between kinase and phosphatase activities in excitable cardiomyocytes.

Objective—We tested the relationship between myocyte kinase/phosphatase imbalance and cellular and whole animal arrhythmia phenotypes associated with ankyrin-B cardiac syndrome.

Methods—Using a combination of biochemical, electrophysiological, and in vivo approaches, we tested the ability of CaMKII inhibition to rescue imbalance in kinase/phosphatase pathways associated with human ankyrin-B-associated cardiac arrhythmia.

Results—The cardiac ryanodine receptor (RyR₂), a validated target of kinase/phosphatase regulation in myocytes, displays abnormal CaMKII-dependent phosphorylation (pS2814 hyperphosphorylation) in ankyrin-B^{+/-} heart. Notably, RyR₂ dysregulation is rescued in myocytes from ankyrin-B^{+/-} mice overexpressing a potent CaMKII-inhibitory peptide (AC3I) and aberrant

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Conflict of Interest/Disclosure: MEA is a cofounder of Allosteros Therapeutics and an inventor on patents claiming to treat heart failure and arrhythmias by CaMKII inhibition. Other authors report no potential conflicts.

RyR₂ open probability observed in ankyrin-B^{+/-} hearts is normalized by treatment with the CaMKII inhibitor KN-93. CaMKII-inhibition is sufficient to rescue abnormalities in ankyrin-B^{+/-} myocyte electrical dysfunction including cellular afterdepolarizations, and significantly blunts whole animal cardiac arrhythmias and sudden death in response to elevated sympathetic tone.

Conclusions—These findings illustrate the complexity of the molecular components involved in human arrhythmia and define regulatory elements of the ankyrin-B pathway in pathophysiology. Furthermore, the findings illustrate the potential impact of CaMKII-inhibition in the treatment of a congenital form of human cardiac arrhythmia.

Keywords

ankyrin; CaMKII; ryanodine receptor; spectrin; arrhythmia

Introduction

Cardiovascular disease is the leading cause of death in the United States, resulting in more than a third of all annual deaths.¹ Moreover, cardiovascular complications account for an estimated \$40 billion in direct and indirect healthcare costs in the United States.¹ Over half of heart disease fatalities are due to cardiac electrical and/or structural defects; therefore understanding the mechanisms underlying potentially fatal cardiac arrhythmias is paramount for uncovering new therapies.

Ankyrins play key roles in cardiac structural and electrical regulation. Three ankyrin genes encode ankyrin polypeptides (ankyrin-R, -B, and -G) with specific functions in cardiac physiology. Ankyrin-R isoforms are linked with organization and regulation of the sarcoplasmic reticulum (SR) and structural proteins obscurin and titin.^{2, 3} Ankyrin-G polypeptides target voltage-gated Na⁺ channels to myocyte intercalated disc membranes and dysfunction in ankyrin-G pathways is linked with the Brugada Syndrome.⁴⁻⁷ Finally, ankyrin-B is found at cardiac transverse-tubule/SR junctions and regulates the local organization of multiple structural and electrical proteins.⁸ Ankyrin-B dysfunction is linked to acquired and congenital forms of human arrhythmia including sinus node disease, atrial fibrillation, ventricular tachycardia, and sudden cardiac death.⁹⁻¹³

Individuals harboring ankyrin-B loss-of-function mutations and ankyrin-B deficient mice display catecholamine-induced ventricular arrhythmia (CPVT).^{12, 13} However, the multifunctional nature of ankyrin-B complicates the process of linking ankyrin-B dysfunction to specific molecular targets in disease. Previously, we identified defects in ankyrin-B dependent targeting of Na/K ATPase and Na/Ca exchanger as important in providing a pro-arrhythmic substrate *in vivo*.¹⁴⁻¹⁶ However, strategies to define additional molecular events that support ankyrin-B-dependent arrhythmia in response to increased sympathetic tone have been unsuccessful. In this study, we utilized a combination of biochemical, electrophysiological, and *in vivo* approaches to identify a role for kinase/phosphatase imbalance in ankyrin-based arrhythmia at the levels of the myocyte and whole animal. We show that ankyrin-B deficiency results in ryanodine receptor hyperphosphorylation, and link this altered post-translational modification state with the calcium/calmodulin-dependent kinase (CaMKII). Utilizing AC3I mice that overexpress a potent CaMKII inhibitory peptide¹⁷, we demonstrate that CaMKII inhibition is sufficient to rescue biochemical, cellular, and whole animal defects and is potent at preventing ankyrin-based cellular afterdepolarizations and fatal cardiac arrhythmias. In summary, these findings define new regulatory roles for ankyrin-B in the cardiac myocyte in the regulation of local CaMKII function in the cardiac dyad.

Methods

Electrophysiology

Current recordings were measured by conventional whole-cell patch-clamp technique with an Axon 200B patch-clamp amplifier controlled by a computer using a Digidata 1320A acquisition board driven by pClamp 8.0 software (Axon Instruments, Foster City, CA). Action potentials (APs) were evoked by brief current pulses 1.5–4 pA, 0.5–1 ms. AP duration (APD) was assessed as the time from the AP upstroke to 90% (or 20 and 50%) repolarization to baseline (APD₉₀).¹⁸ APs were recorded using the perforated (amphotericin B) patch-clamp technique in Tyrode's solution (bath) with the pipette filled with (mmol/L): 130 potassium aspartate, 10 NaCl, 10 HEPES, 0.04 CaCl₂, 2.0 MgATP, 7.0 phosphocreatine, 0.1 NaGTP, and amphotericin B 240 µg/mL, with the pH adjusted to 7.2 with KOH.¹⁹ APs were measured at physiological temperature ± 1 µM isoproterenol.²⁰

Statistics

Statistical significance was determined with a paired Student's *t* test (2-tailed) or ANOVA with the Bonferroni post-hoc test, when appropriate, for continuous data. The null hypothesis was rejected for $p < 0.05$. For experiments in Figure 5B, contingency tables were generated and statistical significance was determined by Fisher's exact test. Data for Figure 5C were analyzed via the Mantel-Cox (logrank) test. Statistical analyses were conducted using GraphPad Prism V4 (GraphPad Software Inc., La Jolla, CA) or SigmaPlot.

Animal models

Mouse models included wild-type C57/Bl6 mice, ankyrin-B^{+/-} mice, AC3I mice, and ankyrin-B^{+/-} mice crossed with AC3I mice.

Single channel recordings

Single channel recordings were performed as described.²¹ See Supplemental Information for additional details.

Immunoblotting

Immunoblots were done as described.¹⁶ See Supplemental Information for additional details.

Antibodies

Custom polyclonal anti-RyR₂ pS2808 (1:1000) and anti-RyR₂ pS2814 (1:1000) phosphorylated epitope-specific antibodies were generated using the peptide C-RTRRI-(pS)-QTSQV corresponding to the PKA phosphorylation site at RyR₂ S2808 and peptide CSQTSQV-(pS)-VD corresponding to CaMKII phosphorylation site at RyR₂ S2814, respectively. These custom antibodies have been used extensively and have been well characterized in previous work.²¹ A monoclonal anti-GAPDH (1:5000, Fitzgerald) and a polyclonal anti-RyR₂ antibody (1:1000, Millipore) were also used.

Conscious ECG experiments

ECG recordings of ambulatory animals were obtained using radiotelemetry (DSI) with transmitters implanted subcutaneously and superficial to the peritoneum seven days before recordings. Recording were obtained from mice both at resting conditions, post-exercise and epinephrine injection. For stress tests, animals were run on a treadmill for 1 hour and then injected IP with epinephrine (2 mg/kg). Non-sustained and sustained arrhythmias were identified using standard ECG analysis guidelines.²² Based on prior data linking significant arrhythmia and sudden cardiac death in the ankyrin-B^{+/-} mouse model¹³, we limited

experiments to a minimal number necessary to obtain statistical power. Arrhythmia was defined as sustained if >2 sec.¹³

Adult cardiomyocyte preparations

Adult cardiomyocytes were prepared as previously described.²³ Murine hearts were obtained after animals were euthanized by acute CO₂ asphyxiation followed by cervical dislocation in accordance with the Guide for the Care and Use of Laboratory Animals published by the NIH and IACUC approved protocols (Ohio State University).

Results

Ankyrin-B deficient mice display RyR₂ hyperphosphorylation

Based on the role of ankyrin-B in regulation of protein phosphatase 2A targeting and regulation in the myocyte transverse-tubule/sarcoplasmic reticulum dyad,^{24, 25} we hypothesized that aberrant control of local phosphorylation events contributes to ankyrin-B cardiac disease. The cardiac ryanodine receptor (RyR₂) is critical for sarcoplasmic reticulum (SR) calcium release, and RyR₂ dysfunction is linked with multiple forms of congenital and acquired cardiac pathologies including exercise- and stress-induced ventricular arrhythmias, atrial fibrillation, and heart failure.²⁶⁻²⁸ Importantly, defects in RyR₂ phosphorylation status are linked with abnormal diastolic SR calcium release and arrhythmia.²⁷⁻²⁹ We first tested whether total RyR₂ expression levels were altered in ankyrin-B^{+/-} (display ~50% reduction in ankyrin-B) mouse hearts by immunoblot. As shown in Figure 1, we observed no statistical difference in total RyR₂ expression in heart lysates prepared from age- and sex-matched wild-type and ankyrin-B^{+/-} mice (Figure 1; 1.0 vs. 1.04; p=N.S.). We next evaluated the phosphorylation status of the cardiac RyR₂ by immunoblot using antibodies directed against two identified RyR₂ phosphorylation sites: S2808, previously linked with protein kinase A (PKA) regulation of RyR₂ open probability (Po) and function,³⁰ and S2814, a regulatory site for the calcium/calmodulin-dependent kinase II (CaMKII³¹). We did not observe statistical differences in RyR₂ S2808 phosphorylation between wild-type and ankyrin-B^{+/-} cardiac lysates (Figure 1; 1.0 vs. 1.25; p=N.S.). In contrast, RyR₂ S2814, was hyperphosphorylated in ankyrin-B^{+/-} cardiac lysates compared with wild-type mouse heart lysates (Figure 1; 1.0 vs. 1.79; p<0.05.). These data suggest that ankyrin-B^{+/-} hearts display aberrant CaMKII-dependent regulation of cardiac RyR₂.

CaMKII is directly linked with abnormal RyR₂ phospho-regulation in ankyrin-deficient hearts

CaMKII-dependent phosphorylation of RyR₂ is directly linked with catecholamine-triggered arrhythmias.²⁹ Therefore, we evaluated whether RyR₂ hyperphosphorylation in ankyrin-B^{+/-} hearts could be directly linked to CaMKII-dependent activity. We assessed the direct role of CaMKII by utilizing AC3-I mice, a transgenic mouse model that overexpresses a potent inhibitor of the CaMKII enzyme in cardiac myocytes.¹⁷ We compared the phosphorylation status of RyR₂ in wild-type mice, ankyrin-B^{+/-} mice, and ankyrin-B^{+/-} 2 crossed with AC3I mice (ankyrin-B^{+/-} X AC3I). We observed a striking reduction in RyR₂ pS2814 phosphorylation in ankyrin-B^{+/-} X AC3I mouse hearts compared with hearts from ankyrin-B^{+/-} littermates (Figure 2, p<0.05). In fact, RyR₂ pS2814 levels in ankyrin-B^{+/-} x AC3I mouse hearts were not significantly different from wild-type mouse hearts (Figure 2, p=N.S.). We observed no difference in total RyR₂ levels between the three genotypes (Figure 2, p=N.S.). These data support a direct link between RyR₂ S2814 hyperphosphorylation and CaMKII in ankyrin-B^{+/-} mice.

CaMKII inhibition rescues ankyrin-based cardiac electrical dysfunction

As noted above, ankyrin-B^{+/-} mice display electrical dysfunction and patients with ankyrin-B loss-of-function mutations display QT_c abnormalities and arrhythmia susceptibility.¹⁴⁻¹⁶ Moreover, ankyrin-B^{+/-} myocytes display severe defects in myocyte electrical function and afterdepolarizations.^{13, 14} Based on our biochemical data, we tested whether CaMKII inhibition was sufficient to prevent electrical dysfunction in primary ankyrin-B^{+/-} adult ventricular cardiomyocytes. We observed mild prolongation of APD₉₀ in ankyrin-B^{+/-} myocytes compared with wild-type myocytes, consistent with QT_c interval changes in specific kindreds harboring *ANK2* loss-of-function variants¹³ (Figure 3A-B; 0.5 Hz, $p < 0.05$). Changes in APD₉₀ in ankyrin-B^{+/-} myocytes were exaggerated in response to adrenergic stimulation (Figure 3A-B, $p < 0.05$) and as expected¹³ we observed significant afterdepolarizations in ankyrin-B^{+/-} myocytes (Figure 3C, $p < 0.05$). In contrast, we observed normalized APD₉₀ in ankyrin-B^{+/-} x AC3I mouse myocytes at baseline compared with myocytes from ankyrin-B^{+/-} littermates (Figure 3A-B, $p < 0.05$). Similar trends were observed for APD₂₀ and APD₅₀ for ankyrin-B^{+/-} x AC3I mice compared with ankyrin-B^{+/-} mice (Supplemental Figure 1). Furthermore, ankyrin-B^{+/-} x AC3I mouse myocytes were resistant to isoproterenol-induced APD prolongation seen in wild-type or ankyrin-B^{+/-} myocytes (Figure 3A-B, $p < 0.05$). Most notably, we observed complete inhibition of cellular afterdepolarizations in isoproterenol treated ankyrin-B^{+/-} x AC3I mice compared with ankyrin-B^{+/-} myocytes (Figure 3C, $p < 0.05$). In summary, our findings demonstrate that CaMKII inhibition rescues both RyR₂ pS2814 hyperphosphorylation and pro-arrhythmic cellular phenotypes in ankyrin-B deficient mice.

Increased ankyrin-B^{+/-} RyR₂ open probability is rescued by KN-93 treatment

Based on altered RyR₂ phosphorylation status in ankyrin-B^{+/-} mice, we next tested whether ankyrin-B^{+/-} mouse hearts displayed abnormal RyR₂ open probability compared to wild-type mouse heart. Consistent with our biochemistry findings, RyR₂ channels from ankyrin-B^{+/-} cardiac SR membrane vesicles²¹ displayed a significant increase in open probability (Po) compared with wild-type mice (Figure 4A-B; 0.154 ± 0.043 vs. 0.007 ± 0.004 , $p < 0.05$). Notably, application of the CaMKII inhibitor KN-93 (10 μ M) significantly reduced RyR₂ Po in ankyrin-B^{+/-} preparations from 0.176 ± 0.039 to 0.099 ± 0.027 (Figure 4C, 4 channels from 3 mice, $p < 0.05$). In contrast, KN-93 did not alter RyR₂ Po in wild-type mice (Figure 4C, 0.011 ± 0.008 vs. 0.006 ± 0.005 , 3 channels from 3 mice; $p = \text{N.S.}$). Collectively, these data support the role of CaMKII-dependent RyR₂ phosphorylation in ankyrin-B^{+/-} electrical phenotypes.

CaMKII inhibition protects against ankyrin-based fatal cardiac arrhythmias

Individuals harboring ankyrin-B loss-of-function mutations display a host of cardiac phenotypes often linked with adrenergic dysregulation.^{11-13, 32} Ankyrin-B^{+/-} mice are haploinsufficient and display similar catecholamine-based arrhythmia.¹³ Therefore, we tested whether CaMKII inhibition could protect the *in vivo* ankyrin-B^{+/-} human disease model from life threatening arrhythmia. To assess arrhythmia susceptibility, we compared ECGs from conscious mice following surgical implantation of radiotelemetry devices. This protocol provides the ability to monitor cardiac activity at physiological heart rates (compared with anesthetized mice) as well as compare ECG parameters following physiological (exercise) or pharmacological intervention. Compared with wild-type mice, ankyrin-B^{+/-} mice subjected to a catecholamine stress protocol (exercise plus intraperitoneal injection of an adrenergic receptor agonist) displayed consistent and severe polymorphic ventricular arrhythmia including ventricular tachycardia/torsade de pointes followed by severe bradycardia and ultimately death (Figure 5A-C). In contrast, we observed no episodes of sustained arrhythmia (Figure 5A-B, 0/6) and no death (Figure 5C, 0/6;). Ankyrin-B^{+/-} mice crossed with AC3I mice subjected to the identical protocol, while showing short

intermittent (<2 sec) non-sustained arrhythmia events, lacked significant sustained arrhythmia phenotypes compared with ankyrin-B^{+/-} mice (Figure 5A-B, p<0.05). Similarly, *in vivo* CaMKII inhibition rescued survival following the cardiac stress protocol (Figure 5C, p<0.05 vs. ankyrin-B^{+/-}) in wild-type or AC3I animals. In conclusion, our data link ankyrin-deficiency with cardiac RyR₂ hyperphosphorylation and abnormal electrical dysfunction at the level of the single cell and whole animal. Moreover, our studies demonstrate that CaMKII inhibition blocks RyR₂ hyperphosphorylation, and rescues single cell afterdepolarizations and arrhythmias.

Discussion

Protein kinase-dependent regulation of cardiac signaling is essential for normal physiology and excitation contraction coupling. Both congenital and acquired forms of human cardiovascular disease are now clearly linked with an imbalance in the kinase/phosphatase axis and cellular dysfunction. In fact, catecholaminergic polymorphic ventricular tachycardia, a disease directly linked with elevated sympathetic tone, exemplifies the striking requirement of precise adrenergic regulation for control of cardiac automaticity.^{33, 34} Beta-adrenergic receptor blockers remain the mainstay for treatment of CPVT patients. However, emerging work from both human and animal models suggests that the protection provided by beta-blockers against potentially lethal arrhythmias is incomplete, demonstrating the need to identify new molecular therapeutic targets for this disease.^{34, 35} Our data link human ankyrin-B-based arrhythmia phenotypes with alterations in the CaMKII signaling pathway. We identify aberrant RyR₂ pS2814 levels in animal models of cardiac ankyrin-B syndrome. Moreover, our data demonstrate that biochemical, cellular, and whole animal phenotypes that are linked with a pro-arrhythmic trigger are either reduced or eliminated in the presence of an *in vivo* cellular CaMKII inhibitory peptide. These studies not only provide important new data regarding the molecular components involved in human arrhythmia, but also define important new regulatory elements of the ankyrin-B pathway for normal physiology.

One interesting finding in this story is the apparent link between ankyrin-B and the kinase/phosphatase axis in the cardiac myocyte. Protein phosphatase 2A (PP2A) is an important negative regulatory factor for both PKA and CaMKII-dependent phosphorylation in heart. The PP2A holoenzyme contains three subunits (A, B, and C), that serve phosphatase targeting, catalytic, and regulatory functions. Notably, we previously demonstrated that ankyrin-B directly associates with B56α, a PP2A regulatory subunit that is highly expressed in heart at the cardiac transverse-tubule.^{24, 25} Moreover, ankyrin-B deficient myocytes display lack of normal B56α targeting presumably that is at least partially responsible for the alterations in RyR₂ phosphorylation observed in this study. Notably, work from Terentyev and colleagues previously showed that miR-1-targeted loss of B56α in myocytes results in CaMKII-dependent hyperphosphorylation of RyR₂ resulting in altered calcium handling and pro-arrhythmic electrical function.³⁶ Together, these findings support a model in which ankyrin-B supports a key role in maintaining local kinase/phosphatase balance in the cardiac dyad by strategically positioning populations of PP2A near important targets including RyR₂ (Figure 6). Thus loss of ankyrin-B-dependent targeting of dyadic PP2A results in increased RyR₂ phosphorylation. It will be important in future experiments to determine whether PP2A activity is reduced and define additional cardiac targets of ankyrin-B-associated PP2A.

CaMKII inhibition has been widely reported as a potential therapeutic strategy for the prevention of heart failure and arrhythmia.³⁷ In fact, relevant for this study, Priori and colleagues recently demonstrated the ability of CaMKII inhibition for protection against arrhythmia in an animal model of CPVT associated with human RyR₂ mutation.³⁸ While our

data link CaMKII inhibition to a reduction in RyR₂ hyperphosphorylation, inhibition of afterdepolarizations, and protection from cardiac arrhythmia the direct mechanisms underlying these phenotypes is clearly complex and likely due to both direct and indirect phosphorylation events. While CaMKII targets RyR₂ regulation, this multifunctional enzyme tunes the activity of a host of regulatory pathways including membrane proteins, transcriptional and metabolic pathways, as well as key SR proteins.³⁷ Thus, additional investigations beyond the scope of this study will be required to uncover the detailed calcium-based regulatory mechanisms altered in this incredibly complex human disease.

Limitations

There are a number of important limitations to our study. First, while our data implicate the CaMKII regulatory pathway in ankyrin-B cardiomyocyte function, we acknowledge that dysfunction in other key molecular components are involved in ankyrin-B cardiac phenotypes. For example, work from our group and others have demonstrated that ankyrin-B targets and regulates a number of key ion channels and transporters in the heart including the Na/Ca exchanger, Na/K ATPase, and Kir6.2-all contributing important roles in cardiac regulation at baseline and in disease.^{13, 16, 39-41} In fact, we predict that loss of specific transverse-tubule populations of Na/Ca exchanger and Na/K ATPase contribute to the pro-arrhythmic substrate by altering local Na⁺ and Ca²⁺ concentration gradients similar to the actions of cardiac glycosides. In fact, we predict that these cellular phenotypes, which ultimately raise SR calcium load, provide the substrate for the arrhythmias triggered by altered adrenergic and CaMKII balance observed in our current study and others.

A second key limitation is the relative role of the cardiac RyR₂ for arrhythmia susceptibility related to CaMKII function in our study. As observed for the cardiac RyR₂ and voltage-gated Na⁺ channel, multiple post-translational regulatory sites likely create an integrated “tuning” rheostat to control ion channel function and thus response to the immediate cellular environment. While our work has assessed RyR₂ S2808 and S2814 sites, these sites are not used to define the critical target for regulation, but instead provide a physiologically relevant surrogate of CaMKII function in heart. We predict that other CaMKII downstream proteins will also play critical roles in the ankyrin-B phenotype in response to altered sympathetic tone.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Altered RyR₂ phosphorylation in ankyrin-B^{+/-} hearts

A) Representative immunoblots indicating expression of Total RyR₂, RyR₂ pS2808, and RyR₂ pS2814 in wild-type and ankyrin-B^{+/-} hearts. For all experiments, protein values were normalized against internal loading control (GAPDH). B-D) Densitometry analysis indicating the relative levels of total RyR₂ (B), RyR₂ pS2808 (C), and RyR₂ +/- pS2814 (D) expression in WT and ankyrin-B hearts (N values are noted in each panel). In panel D, * denotes $p < 0.05$.

Figure 2. CaMKII inhibition normalizes RyR₂ hyperphosphorylation in ankyrin-B^{+/-} hearts
 A) Representative immunoblots showing levels of RyR₂ pS2814 in wild-type, ankyrin-B^{+/-}, and ankyrin-B^{+/-} X AC3I hearts. Total RyR₂ levels were unchanged between the three genotypes studies (p=N.S.). For all experiments, protein values were normalized against internal loading control (GAPDH). Line between lanes in A denotes that data collected from non-contiguous lanes of same gel. B) Densitometric analysis of RyR₂ pS2814 in wild-type, ankyrin-B^{+/-}, and AC3I x ankyrin-B^{+/-} animals after treatment with isoproterenol. N values are listed in panel and * represents p<0.05 for wild-type vs. ankyrin-B^{+/-} and ankyrin-B^{+/-} vs. ankyrin-B^{+/-} X AC3I.

Figure 3. CaMKII inhibition normalizes ankyrin-B^{+/-} myocyte electrical phenotypes

A) Action potential morphology in wild-type, ankyrin-B^{+/-}, AC3I, and ankyrin-B^{+/-} X AC3I myocytes ± isoproterenol (1 μM). Red arrow indicates after-depolarization. Note difference in time scales for each genotype. B) Action potential duration (APD₉₀) of wild-type, ankyrin-B^{+/-}, AC3I, and ankyrin-B^{+/-} X AC3I cardiomyocytes paced at 0.5 Hz at baseline (black bars) or in the presence of beta-adrenergic agonist (red bars). In B, * denotes p<0.05 vs. each respective β-AR stimulation group; # denotes p<0.05 vs. each respective wild-type group; and Δ denotes p<0.05 vs. each respective ankyrin-B^{+/-} group. C) CaMKII inhibition normalizes ankyrin-B^{+/-} myocyte afterdepolarizations. Bars denote incidence of myocyte afterdepolarizations ± isoproterenol treatment. In C, * denotes p<0.05 vs. each respective β-AR stimulation group and # denotes p<0.05 vs. each respective wild-type group. For B and C, N values are noted in each panel.

Figure 4. Ankyrin-B^{+/-} mouse hearts display increased RyR₂ open probability (Po)

A) Representative RyR₂ single channel recordings from lipid bilayers prepared from wild-type and ankyrin-B^{+/-} mouse hearts. B) Average Po of RyR₂ from wild-type and ankyrin-B^{+/-} mice. Po was increased in ankyrin-B^{+/-} group (7 channels from 3 mice) compared to wild-type group (6 channels from 3 mice). C) Effect of KN-93 on Po of RyR₂ channels. Application of CaMKII inhibitor KN-93 reduced Po of RyR₂ from ankyrin-B^{+/-} hearts, but did not alter Po of RyR₂ from WT mouse hearts. Numbers in each bar represent number of channels (number of mice) in the bar graph (* denotes $p < 0.05$).

Figure 5. CaMKII inhibition normalizes ankyrin-B^{+/−} arrhythmia phenotypes

A) Representative ECG recordings from wild-type, ankyrin-B^{+/−}, AC3I, and ankyrin-B^{+/−} X AC3I mice at baseline and following exercise and treatment with adrenergic agonist. Center recording for ankyrin-B^{+/−} mouse illustrates typical example of arrhythmia lasting minutes from this mouse model. B) Incidence of sustained ventricular arrhythmia in wild-type, ankyrin-B^{+/−}, AC3I, and ankyrin-B^{+/−} X AC3I mice following exercise and treatment with β -adrenergic agonist. In panel B, # denotes $p < 0.05$ for ankyrin-B^{+/−} mice vs. wild-type mice and Δ denotes $p < 0.05$ for ankyrin-B^{+/−} mice vs. ankyrin-B^{+/−} X AC3I mice. C) Survival of wild-type, ankyrin-B^{+/−}, AC3I, and ankyrin-B^{+/−} X AC3I mice following exercise and treatment with adrenergic agonist. In panel C, * denotes $p < 0.05$ of ankyrin-B^{+/−} vs. wild-type mice. For B-C, N values are listed in panels.

Figure 6. Model for link between ankyrin-B and local sympathetic regulation

In myocytes, ankyrin-B targets local PP2A activity through an interaction with B56 α , one regulatory subunit of the PP2A holoenzyme. When ankyrin-B function is altered, this targeting mechanism is disrupted allowing CaMKII to function unopposed on myocyte targets (including RyR2) resulting in electrical dysfunction.