Cardiac arrhythmias are a major epidemiological and public health problem and contribute significantly to sudden cardiac death, heart failure, stroke, suffering, debilitation, and healthcare expenses. In the United States alone, sudden cardiac death is estimated to kill 250,000 to 400,000 people annually.1 Most sudden death is due to cardiac arrhythmias,2 with ventricular tachycardia and fibrillation as the most commonly (~80%) recorded rhythms in out-of-hospital cardiac arrests.3 In patients with structural heart disease, mostly resulting from a history of myocardial infarction, arrhythmias are the main cause of death.4 Atrial fibrillation (AF) and sinus node dysfunction (SND) are the most common sustained arrhythmias.5 AF affects ~2.3 million patients in the United States,6 and because the prevalence of AF increases with age, it is predicted to increase by 2.5-fold by 2050.6 Patients with AF have approximately twice the mortality rate of patients in sinus rhythm,6 and the incidence of stroke is increased by 2- to 7-fold.7 AF is a costly disease and causes a public health burden estimated at $6.0 to $26.0 billion annually in the United States.8 SND is associated with increased sudden cardiac death, particularly in patients with heart failure, and a large portion (~40%) of mortality in hospitalized patients with heart failure may be secondary to SND.9 SND is the indication for 60% of the 180,000 pacemakers implanted in the United States each year, a procedure that in 2004 accounted for ~$2 billion in expenses.10,11 The negative impact of arrhythmias on human health and medical economics is a major motivating factor for establishing new and effective therapeutic approaches.

Cardiac arrhythmias are the result of cell membrane hyperexcitability (the cause of automatic and triggered tachyarrhythmias), defective impulse formation (the cause of SND), or reduction in normal cell-to-cell electrical coupling (the cause of conduction system “block” and a component of the zone of slow conduction in most arrhythmias supported by a reentrant circuit). Ion channels, macromolecular protein complexes with a cell membrane-spanning conductance pathway, are the fundamental units of membrane excitability, and rare congenital defects in ion channels or proarrhythmic off-target actions of many drugs can be sufficient to promote arrhythmia risk. However, most arrhythmias are not attributable to monogenic defects or drugs and occur in the biological context of various proarhythmic factors such as advanced age, increased oxidant stress, ischemia, tissue injury, inflammation, and systemic disease (eg, hypertension, diabetes, heart failure). These proarhythmic factors appear to favor structural remodeling of cardiac tissue and to predispose certain ion channels to initiate or sustain arrhythmias. Unfortunately, ion channel antagonist drugs have not proved to be broadly applicable, safe, or effective antiarrhythmic agents.12,13 Thus, a major goal for science and industry is to define molecular pathways and mechanisms that cause common and life-threatening arrhythmias to develop new and improved therapies. The multifunctional Ca2+/calmodulin-dependent protein kinase II (CaMKII) has emerged as a highly validated molecular mechanism with the potential to connect “upstream” proarhythmic factors such as oxidation with “downstream” responses such as ion channel hyperactivity, defective intracellular Ca2+ homeostasis, tissue damage, and scar formation that promote arrhythmias. Here, we review modern concepts of CaMKII molecular physiology in the context of fundamental arrhythmia mechanisms and consider evidence that CaMKII inhibition could be a broad-spectrum antiarrhythmic strategy.

A Brief Overview of Arrhythmia Mechanisms

To move forward with this review, we have decided to pause and put forth a parsimonious but global concept for understanding arrhythmias. Although there will be exceptions that appear to violate this framework, they will be unusual and outside the experience of most clinical practice. Most consequential arrhythmias cause heart rates that are too fast or too slow for optimal mechanical performance of the heart. Arrhythmias can be relatively regular (ie, occur at a constant period) or irregular. Fast (tachy) rhythms arise from 1 or more of 3 basic mechanisms (Figure 1). Triggered arrhythmias are the result of enhanced cell membrane excitability caused by an imbalance in currents that favors excessive net inward current. The inward current depolarizes the cell membrane, causing a positive deflection in the membrane potential called an afterdepolarization. Early afterdepolarizations (EADs) occur during action potential depolarization, and delayed afterdepolarizations (DADs) interrupt the diastolic interval after action potential repolarization is complete. EADs and
DADs trigger arrhythmias when they depolarize the cell membrane to the threshold for action potential initiation. Current evidence suggests that some forms of AF, ventricular tachycardia, and ventricular fibrillation may be initiated and sustained by EADs and DADs.\(^{14}\) Automatic arrhythmias typically arise in cardiomyocytes associated with the pacemaking or specialized conduction system, including cells in the pulmonary outflow track and in pulmonary vein myocytes that are linked to some forms of ventricular tachycardia, atrial tachycardia, and AF. Automatic and triggered arrhythmias are fundamentally similar in that both depend on cell membrane hyperexcitability, which is driven primarily by Ca\(^{2+}\) and/or adrenergic receptor stimulation. Reentry refers to an arrhythmia substrate that permits a repetitively excitable circuit, which may be anatomically constituted, eg, by fibrosis, or functional, eg, by electric refractoriness caused by action potential prolongation, membrane depolarization, or tissue gradients of activation and/or repolarization. Stable reentrant circuits require an excitable gap, a zone of slow conduction, and a preferred direction for conduction, as may occur with unidirectional block. Reentry is the basis for common forms of supraventricular tachycardia (including typical atrial flutter, atrioventricular nodal reentrant tachycardia, and accessory pathway–mediated tachycardias) and ventricular tachycardia (particularly in the setting of structural heart disease). Triggered and reentrant mechanisms likely coexist, particularly in structurally diseased tissue. Slow (brady) arrhythmias also may be due to conduction slowing or unidirectional conduction block, eg, as occurs in atrioventricular conduction disturbances or “block.”

SND results from defective impulse formation caused by intrinsic or acquired defects in depolarizing current, loss of sinoatrial nodal pacemaker cells, or inherited or acquired impairment of conduction. Remarkably, excessive activation of CaMKII is implicated in tachyarrhythmias and bradyarrhythmias by each of these mechanisms. The surprising breadth of CaMKII participation in arrhythmias appears to be rooted in the role of CaMKII signaling to ion channels and the involvement of excessively activated CaMKII in promoting cell death and fibrosis.

**CaMKII Molecular Physiology**

CaMKII structure determines CaMKII function, and the molecular physiology of CaMKII is ideal for connecting upstream signals encoded in intracellular Ca\(^{2+}\) and oxidation into downstream events that support core physiological functions in the cardiovascular system such as fight-or-flight heart rate increases and excitation-contraction coupling. However, posttranslational modifications can convert CaMKII from a Ca\(^{2+}\)/calmodulin-dependent enzyme to a Ca\(^{2+}\)/calmodulin-independent enzyme that plays a role in diverse forms of cardiovascular disease, including arrhythmias. CaMKII is a multifunctional serine-threonine protein kinase with widespread expression in muscle, nerve, and immune tissues. The role of CaMKII and other protein kinases is to lower the

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**Figure 1.** The role of Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII) in normal heart (top) and CaMKII-linked proarrhythmias in structural heart disease (bottom). The normal system for excitation-contraction coupling (ECC) and conduction in the heart leads to sinus rhythm, as detected by the surface ECG (top left). On the single-cardiomyocyte level (top right), excitation opens voltage-gated Na\(^+\) channels responsible for Na\(^+\) current (I\(_{Na}\)) leading to depolarization and triggering voltage-gated L-type Ca\(^{2+}\) current (I\(_{Ca}\)) to initiate myofilament cross-bridge formation, which supports contraction by stimulating ryanodine receptors (RyRs) to release Ca\(^{2+}\) from the sarcoplasmic reticulum (SR). Relaxation occurs mainly by Ca\(^{2+}\) uptake to the SR by phospholamban (PLB)-regulated sarcoplasmic-endoplasmic reticulum Ca\(^{2+}\) ATPase (SERCA2a) and extrusion to the extracellular space by the Na\(^+\)/Ca\(^{2+}\) exchanger (NCX). CaMKII is an integral part of ECC and orchestrates all of its key components (arrows, right). The sarcolemmal currents shape the action potential (inset, middle). In structural heart disease (bottom), overexpressed and activated CaMKII is further activated by oxidation through an aldosterone- and angiotensin II (Ang II)–dependent increase in reactive oxygen species (ROS). In this setting, CaMKII disturbs Ca\(^{2+}\) homeostasis by hyperphosphorylating Ca\(_{1.2}, Na_{1.5},\) and RyRs, leading to increased intracellular Ca\(^{2+}\) (Ca\(^{2+}\)\(_{i}\)) and early and late afterdepolarizations (EADs, DADs; right and inset, middle). Afterdepolarizations can initiate and sustain arrhythmias, including atrial and ventricular premature complexes and atrial fibrillation (AF; top left). Increased Ca\(^{2+}\)\(_{i}\) causes mitochondrial death and

CaMKII Molecular Physiology

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**Figure 1 (Continued).** Apoptosis, leading to scarring and reparative fibrosis (bottom right), which promote conduction slowing and reentry circuits (ventricular tachycardia [VT]; left middle). Similarly, in the sinus node, CaMKII activation causes apoptosis of pacemaker cells, leading to decreased impulse formation and sinus node dysfunction (SND; bottom left).
free-energy barrier and thus markedly hasten (ie, catalyze) the reaction rate for transferring the terminal phosphate (γ phosphate) of ATP to a serine or threonine, which is part of a consensus sequence, on a target protein. Phosphorylation is a fundamental mechanism for biological systems to rapidly change the rate or function of many types of molecules (ie, proteins and lipids). Ion channel proteins and Ca\(^{2+}\) homeostatic proteins involved in excitation-contraction coupling are CaMKII targets of immediate importance to arrhythmias. Protein phosphorylation is reversible by protein phosphatases, enzymes that catalyze the removal of phosphate adducts from proteins. Although they are important and functionally complementary to kinases, a discussion of phosphatases is beyond the scope of this review format. The "rules" for the CaMKII consensus sequence are not inviolate. However, in general, CaMKII prefers to catalyze phosphorylation within an RXXS/T motif, where R is arginine, X is any amino acid, S is a serine, and T is a threonine. We can use the RXXS/T sequence motif as a guide to identifying candidate CaMKII "sites." There are 4 different CaMKII genes, and each gene encodes a distinct CaMKII isoform (α, β, γ, δ). All CaMKII isoforms appear to share common regulatory mechanisms and protein targets but differ in tissue distribution. CaMKIIδ is abundant in myocardium, and recent studies using knockout mice lacking CaMKIIδ have shown resistance to myocardial hypertrophy and heart failure after aortic banding surgery, exhibiting reduced proarrhythmic intracellular Ca\(^{2+}\) release events, and are resistant to vascular injury, validating the concept that CaMKIIδ can promote cardiovascular disease, including arrhythmias and sudden death. CaMKIIδ has multiple splice variants (ie, specific molecular sequences are determined by exon skipping, a process of making "choices" about which particular exons to translate and which to leave untranslated), including 2 variants with the potential to guide subcellular localization of the CaMKII holoenzyme (see below).

CaMKII assembles into a dodecameric holoenzyme built from a pair of stacked hexamers. Each CaMKII monomer consists of a core regulatory domain bound by an N-terminus catalytic domain and a C-terminus association domain (Figure 2A). Like other kinases, the catalytic domain has an ATP-binding pocket that creates a microenvironment to lower the energy required to hydrolyze ATP, enhancing the rate of transfer for the terminal phosphate (γ-phosphate) from ATP to a target S/T residue (Figure 2B). CaMKII is enzymatically inactive because the catalytic domain is constrained by the AI sequence on the regulatory domain. CaMKIIδ has a C-terminus association domain (right) and an N-terminus regulatory domain. Oxidation at Met281/282 or auto-phosphorylation at Thr287 prevents reassociation of the catalytic domain by disabling the AI sequence, leading to constitutive, Ca\(^{2+}\)/CaM-independent CaMKII activity. The CaMKII holoenzyme is a dodecamer, assembled from CaMKII monomers as a stacked pair of hexameric rings.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** The domain structure of a Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII). A, Each CaMKII monomer has a C-terminus association domain (right) and an N-terminus catalytic domain (left). The internal regulatory domain consists of a C-terminus Ca\(^{2+}\)/CaM binding region (CaM-B) and an N-terminal side autoinhibitory region (AI; top). When inactive, the catalytic domain is constrained by the AI sequence on the regulatory domain (bottom right). Oxidation at Met281/282 or auto-phosphorylation at Thr287 prevents reassociation of the catalytic domain by disabling the AI sequence, leading to constitutive, Ca\(^{2+}\)/CaM-independent CaMKII activity. B, The CaMKII holoenzyme is a dodecamer, assembled from CaMKII monomers as a stacked pair of hexameric rings.

tant effects on the transcriptional activity of genes involved in muscle hypertrophy, whereas CaMKIIδ\(_{C}\) splice variants in the cytoplasm have a prominent role in regulating membrane excitability and intracellular Ca\(^{2+}\) homeostasis. Recently, a CaMKII holoenzyme crystal structure was resolved, revealing that variation in the length of the hypervariable region modulates access of Ca\(^{2+}\)-bound calmodulin (Ca\(^{2+}\)/CaM) to the regulatory domain. Thus, the length of the hypervariable linker appears to be a fundamental structural mechanism for tuning the Ca\(^{2+}\) sensitivity of CaMKII, an aspect of CaMKII molecular pathophysiology with potential but untested implications for the Ca\(^{2+}\) dependence of arrhythmias. Under resting conditions (ie, low redox potential and low Ca\(^{2+}\)), CaMKII is enzymatically inactive because the catalytic domain is bound to an autoinhibitory region embedded within the N-terminus portion of the regulatory domain (Figure 2B). CaMKII is initially activated when an increase in Ca\(^{2+}\) favors Ca\(^{2+}\)/CaM binding to calmodulin (CaM), a ubiquitous intracellular Ca\(^{2+}\)-binding protein. Ca\(^{2+}\)/CaM binds to a CaM-binding region that resides in the C terminus of the CaMKII regulatory domain, leading to a conformational distortion that displaces the autoinhibitory region from the catalytic domain and causes CaMKII to become enzymatically active. Initially, Ca\(^{2+}\)/CaM unbinding reverses CaMKII activation. However, under conditions in which elevated Ca\(^{2+}\), is persistent or reactive oxygen species (ROS) are increased (see below), CaMKII can sustain activity even after Ca\(^{2+}\)/CaM unbinding. Most cardiovascular diseases, including arrhythmias, appear to be associated with excessive Ca\(^{2+}\)/CaM-autonomous activity, so a brief discussion of mechanisms promoting Ca\(^{2+}\)/CaM-independent activity follows.
When Ca\(^{2+}\)/CaM is persistently bound to CaMKII, CaMKII undergoes intersubunit autophosphorylation at Thr287 (the specific numbering varies slightly by isoform). Given the holoenzyme structure of CaMKII, it is intuitive that activated CaMKII monomers bound up in close proximity to a desirable CaMKII target site (ie, Thr287) lead to autophosphorylation. Thr287 autophosphorylation promotes CaMKII activity by 2 processes. First, autophosphorylation increases the affinity of Ca\(^{2+}\)/CaM for CaMKII by 1000-fold, so-called calmodulin trapping. Second, even after Ca\(^{2+}\)/CaM unbinding, the Thr287-autophosphorylated form of CaMKII has residual enzymatic activity because phosphorylation of Thr287 prevents effective reassociation and constraint of the catalytic domain by the autoinhibitory region. Thr287 autophosphorylation is favored by high-frequency Ca\(^{2+}\)/CaM stimulation (ie, as occurs in tachycardia) and prolonged (ie, as occurs in proarrhythmic electric remodeling in heart failure or in the long-QT syndrome) intracellular Ca\(^{2+}\) spikes,\(^{26}\) features that have led to the assertion that CaMKII is a “memory” molecule. In contrast to the activating effects of autophosphorylation at Thr287, autophosphorylation of Thr306 in the CaM-binding region can reduce Ca\(^{2+}\)/CaM-dependent CaMKII activity by decreasing the affinity of CaMKII for Ca\(^{2+}\)/CaM.\(^{27}\) Thus, CaMKII autophosphorylation represents a posttranslational modification with the capacity to tune CaMKII activity and to transform CaMKII into a Ca\(^{2+}\)/CaM-autonomous enzyme.

CaMKII activity is promoted by conditions of increased ROS, as occur in myocardium prone to tachycardia\(^{28,29}\) and bradycardia, owing to SND.\(^{30}\) High-ROS conditions can enhance CaMKII activity by direct and indirect actions. Oxidation may increase the abundance of Thr287-autophosphorylated CaMKII by inactivating phosphatases.\(^{31}\) Our group identified a mechanism by which oxidation of methionine (Met) 281 and Met282 in CaMKII leads to Ca\(^{2+}\)/CaM-autonomous activity by a posttranslational modification that is analogous to Thr287 autophosphorylation (Figure 3).\(^{32}\) Like Thr287 autophosphorylation, Met281/282 oxidation prevents reassociation of the catalytic and autoinhibitory domains even in the absence of Ca\(^{2+}\)/CaM binding, causing constitutive, Ca\(^{2+}\)/CaM-autonomous CaMKII activity. Importantly, oxidation may increase the sensitivity of CaMKII to activation by Ca\(^{2+}\)/CaM,\(^{33}\) suggesting that CaMKII could become proarrhythmic in the setting of increased ROS, even in the absence of increased Ca\(^{2+}\). The first oxidation step (ie, methionine sulfoxide) for Met281/282 oxidation is enzymatically reversible by methionine sulfoxide reductase A (MsrA). MsrA is an interesting reductase, in part because it is implicated as a determinant of lifespan; mice lacking MsrA have shorter lives,\(^{34}\) whereas nonmammalian model organisms (ie, worms and flies) exhibit increased lifespan by MsrA overexpression. Our findings suggest that CaMKII is an important target of MsrA and that MsrA knockout increases susceptibility to myocardial oxidant injury\(^{32,35}\) whereas myocardial MsrA overexpression is protective against oxidant injury.\(^{35}\) At this point, proof-of-concept studies testing whether increasing MsrA activity is antiarrhythmic are lacking. Oxidized CaMKII does not show CaM trapping because oxidation of Met308 reduces the affinity of Ca\(^{2+}\)/CaM binding.\(^{32}\) Thus, the CaMKII holoenzyme is able to detect ROS and the frequency and duration of intracellular Ca\(^{2+}\) signals. The ability of CaMKII to transition into a Ca\(^{2+}\)/CaM-autonomous enzyme appears to be critical to the pathological roles of CaMKII in cardiovascular diseases, including arrhythmias.

**Ion Channels Are Fundamental**

The development of the ECG by Einthoven\(^{36}\) marked the beginning of cardiac electrophysiology and initiated an explosion of mechanistically informed research that contributed to a growing understanding of arrhythmias. A key event in this discovery process was the development of a voltage-clamp technique applicable to single cells (so-called patch clamp) in 1981 (Figure 4).\(^{37}\) Patch clamp allowed very high-resistance seals between a glass micropipette containing an electrode and the cell membrane that was required to resolve tiny currents (10\(^{-10}\)–10\(^{-12}\) A) associated with indi-
Individual ion channels. Scientists armed with new patch-clamp technology rapidly identified discrete ionic currents in heart, leading to a biophysical understanding and naming of many ionic currents that were plausibly linked to cardiac electrophysiology and arrhythmias. The identification of patients with heritable arrhythmias, with eventual linkage to genetic mutations encoding defective ion channel proteins, made it clear that ion channel defects alone, even in the absence of structural heart disease, could be sufficient to induce arrhythmias. The rise of molecular biology allowed investigators to begin to understand how genetic mutations could lead to defective ion channel protein structure, function, and arrhythmias. The use of genetically modified mouse models provided important insights into the effects of ion channel–encoding gene deletion in vivo. Improved high-resolution crystal structures and tools for dynamic imaging of ion channels provide hope that integrated application of complementary experimental approaches will improve our understanding of ion channel biology and the mechanisms by which ion channels contribute to arrhythmias.

**Action Potentials Are Built From Ionic Currents**

Cardiac action potentials initiate each heartbeat and are the result of highly coordinated activity of ion channels. The ventricular myocyte action potential has a long duration (200–400 milliseconds) compared with action potentials in neurons (10 milliseconds) and is divided into numbered phases (Figure 5). In the diastolic interval (phase 4), between action potentials, the inner resting cell membrane potential is negatively charged (90 mV in contracting myocardium and 60 mV in sinoatrial nodal pacemaker cells) compared with the extracellular space. In sinoatrial nodal pacemaker cells, phase 4 is dynamic, exhibiting a pattern of late diastolic depolarization that culminates in action potential initiation. Pacemaker cell membrane diastolic depolarization rate is under autonomic nerve control, and catecholamines increase heart rate by augmenting the inward “funny” current ($I_f$) through cAMP-responsive (gated) channels and by enhancing the electrogenic Na$^+$/Ca$^{2+}$ exchanger current ($I_{NCX}$), which is activated by release of Ca$^{2+}$ from intracellular sarcoplasmic reticulum (SR) ryanodine receptor Ca$^{2+}$ channels. Action potentials in atrial and ventricular myocardial cells are initiated (phase 0) by an inward (positive) Na$^+$

![Figure 4. Simplified schematic of a patch-clamp setup. A thin glass patch pipette (open tip, ~1 μm in diameter) is tightly attached (sealed) to an area of the cardiomyocyte cell membrane (patch). In this cell on configuration, it is possible to record ionic currents from single ion channels. By applying suction, it is possible to break the patch while maintaining the high-resistance seal (whole cell configuration) so that ionic current from all ion channels or a subset of pharmacologically or biophysically selected ion channels in the cell membrane can be measured. The patch pipette includes a pipette solution and a silver wire electrode connected to an operational amplifier. The amplifier measures the voltage (or current) in the micropipette in relation to a reference electrode in the bath solution connected to the ground and injects or withdraws current to maintain a command voltage (voltage clamp).](image)

![Figure 5. Ventricular action potential (AP) and ionic currents. The morphology of a ventricular AP is shaped by 4 phases (top left): phase 0, rapid upstroke, generated by the voltage-gated Na$^+$ current; phase 1, early repolarization, inactivation of Na$^+$ current, and activation of repolarizing transient outward K$^+$ current $I_{to}$; phase 2, plateau, orchestrated by Ca$^{2+}$ currents and repolarizing K$^+$ currents; and phase 3, late repolarization, inactivation of Ca$^{2+}$ currents and sustained activation of K$^+$ currents. In phase 4, diastole, the resting membrane potential is governed mainly by the K$^+$ current $I_{K1}$. A schematic overview of transsarcolemmal ionic movements is shown at the bottom left. Typical ionic current flows that orchestrate a ventricular AP are shown on the right, along with the pore-forming proteins and encoding genes.](image)
current (\(I_{Na}\)), but in pacemaker cells, with less negatively charged cell membranes, \(Na^+\) channels are either absent or inactivated, and action potentials are initiated by inward \(Ca^{2+}\) current (\(I_{Ca}\)) and/or inward \(Na^+/Ca^{2+}\) exchanger current. In ventricular myocytes, at the conclusion of phase 0, the vast majority of \(Na^+\) channels are inactivated, and rapidly activating outward \(K^+\) currents open and sculpt a repolarizing notch in the action potential before the plateau (phase 1).

The cardiac action potential plateau (phase 2) is marked by a relatively low amount of ion movement and high cell membrane resistivity, so small currents have relatively large effects on membrane potential. These features cause the action potential plateau to be particularly vulnerable to arrhythmia-triggering EADs.\(^45\) Action potential repolarization (late phase 2 and phase 3) occurs when inward current wanes and outward currents (mainly owing to \(K^+\)) increase. Action potentials spread across the myocardium as a wave front of depolarization to trigger mechanical systole. The coupling between electric and mechanical events is called excitation-contraction coupling and is accomplished by a \(Ca^{2+}\)-induced \(Ca^{2+}\) release process.\(^46\) Action potentials resolve as a wave back, leaving behind electrically excitable myocardium, poised to generate another action potential.

### Ion Channel Antagonist Drugs Are Suboptimal Agents

The improved understanding of ion channels and the recognition of their role as determinants of cardiac arrhythmia occurred at a time when an increasingly sophisticated pharmaceutical industry became intent on designing molecularly focused drug therapies. Flecainide and encainide, potent and selective \(I_{Na}\) antagonists, were famously evaluated in the Cardiac Arrhythmia Suppression Trial (CAST).\(^13\) CAST was motivated by the fact that patients with structural heart disease (left ventricular ejection fraction \(\leq 30\%\) after myocardial infarction) have a high rate of sudden death caused by arrhythmias. Earlier work had shown that patients with depressed ejection fractions and/or frequent premature ventricular contractions, a potential consequence of afterdepolarizations, were at highest risk for sudden death. The CAST pilot study first established the efficacy of flecainide and encainide in suppressing premature ventricular contractions—by \(\geq 80\%\)—with Holter monitoring. Nonetheless, CAST showed that patients treated with encainide or flecainide were significantly more likely to die suddenly compared with placebo-treated patients, presumably as a result of the proarrhythmic effects of \(Na^+\) channel inhibition. The Survival With Oral d-Sotalol (SWORD) was a second critical study that definitively demonstrated increased mortality in post–myocardial infarction patients with reduced ejection fractions treated with a relatively selective \(K^+\) channel antagonist.\(^12\) Like many drugs, sotalol is a racemic modification in which the \(l\)-enantiomer has \(K^+\) channel antagonist and \(\beta\)-adrenergic receptor antagonist actions. In contrast, the \(d\)-enantiomer lacks activity at \(\beta\)-adrenergic receptors. Patients treated with \(d\)-sotalol had increased mortality compared with control subjects. Taken together, these seminal studies suggested that ion channel antagonist drugs were suboptimal for treating arrhythmias and were potentially dangerous in patients at high risk for life-threatening arrhythmias resulting from structural heart disease.

### The Emergence of Antiarrhythmic Device Therapies

The failure of ion channel–targeted antiarrhythmic drugs in CAST and SWORD occurred during a period of discovery for the long-QT syndromes. In many cases, the defective ion channel–encoding genes in long-QT syndrome patients led to proarrhythmic defects that mimicked the effects of antiarrhythmic drugs, suggesting to many that ion channel–targeted therapy alone was unlikely to be a broadly applicable or successful antiarrhythmic strategy because of the risk of proarrhythmia. The surprising results of CAST marked the beginning of a shift away from ion channel antagonist therapies and toward the use of surgically implanted cardiac defibrillators.\(^37\) Although surgically implanted cardiac defibrillator therapy does not affect biological factors driving the progression of structural heart disease, cardiac resynchronization therapy by biventricular pacing can reverse adverse structural remodeling of failing left ventricles.\(^48\) Studies in dogs show increased myocardial CaMKII activity and apoptosis in the lateral left ventricle in tachypacing-induced heart failure with left bundle-branch ablation that is reversed by cardiac resynchronization therapy.\(^49\) These findings suggest that CaMKII activity may be favorably modulated by biventricular pacing and that normalization of CaMKII activity could account for some of the clinical benefits of cardiac resynchronization therapy.

### Understanding the Proarrhythmic Actions of CaMKII at Ion Channels

CaMKII is now known to orchestrate connections between intracellular \(Ca^{2+}\) and membrane excitability by actions at virtually all known voltage-gated ion channels in heart. It is also likely that CaMKII connects changes in redox potential to cell membrane potential through actions at ion channels.\(^50\) Given the central role of CaMKII in modulating electric activity in heart, it is perhaps not surprising that excessive CaMKII activation favors arrhythmias (see below) in part by actions at ion channels. A comprehensive discussion of CaMKII actions on ion channels is beyond the scope of this review and has been published elsewhere.\(^51\) Instead, we focus on 2 cell membrane (sarcolemmal) ion channels that are prominently represented in ventricular myocardium, voltage-gated \(Ca^{2+}\) (\(CaV_{1.2}\)) and \(Na^+\) (\(Nav_{1.5}\)) channels, in which the role of CaMKII in arrhythmogenesis is relatively well established.\(^28\)\(^52\) In a later section, we also discuss the effects of CaMKII on an intracellular \(Ca^{2+}\) releasing ion channel, the ryanodine receptor, to illustrate important molecular and cellular concepts relevant to clinical arrhythmias.

CaMKII catalyzes phosphorylation of the pore-forming \(\alpha\) subunit of the predominant ventricular myocardial L-type Ca channel, \(CaV_{1.2}\), at Ser1512 and Ser1570 and the accessory \(\beta\) subunit at Thr498.\(^53\)\(^55\) The effect of CaMKII-dependent phosphorylation on whole-cell (ie, macroscopic) \(I_{Ca}\) is to promote a dynamic process called facilitation,\(^56\) which results in enhanced peak and slowed \(I_{Ca}\) inactivation as an initial response to repetitive cell membrane depolarizations (Figure 6). At a single-
CaMKII effects on CaV1.2 may contribute to prolongation of the action potential duration as a consequence of increasing opening. The relatively long cardiac action potential plateau is at least in part because the plateau potential is preferentially during the plateau phase of the cardiac action potential, at least in part because the plateau potential is preferentially during the plateau phase of the cardiac action potential. The relatively long cardiac action potential plateau appears to go hand in hand with proarrhythmic vulnerability. Pathological action potential prolongation is a prominent outcome of a proarrhythmic feed-forward mechanism shared with CaV1.2 openings, so-called mode 2 openings, a process called facilitation, which may also contribute to action potential (AP) prolongation (top). Facilitation is a positive CaMKII-dependent staircase of \( I_{\text{Ca}} \) amplitudes over a series of several APs. CaMKII phosphorylation alters inactivation and activation properties of LTCC current, moving the availability of Ca\(^{2+}\) channels to more positive membrane potentials and the activation to more negative potentials. In this way, CaMKII increases the window current (shaded field between the curves) of the \( I_{\text{Ca}} \), increasing the probability of Ca\(^{2+}\) channel openings and CaMKII activity by increasing Ca\(^{2+}\) entry through \( I_{\text{Ca}} \) and activating CaMKII (Figure 7). Facilitation increases sarcoplasmic (SR) Ca\(^{2+}\) leak and Ca\(^{2+}\)\(_{\text{i}}\), which in turn fuels the Na\(^{+}\)/Ca\(^{2+}\) exchanger (NCX) forward mode, causing delayed afterdepolarizations (DADs). Importantly, CaMKII integrates several proarhythmogenic mechanisms that may work to further augment CaMKII activity by increasing Ca\(^{2+}\)\(_{\text{i}}\) and ROS.

Figure 6. Regulation of L-type Ca channels (LTCC) by Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII). CaMKII phosphorylation of LTCC alters channel gating by increasing open probability. In myocardium, this mechanism leads to a frequency-dependent increase in Ca\(^{2+}\) current \( I_{\text{Ca}} \) (bottom), a process called facilitation, which may also contribute to action potential (AP) prolongation (top). Facilitation is a positive CaMKII-dependent staircase of \( I_{\text{Ca}} \) amplitudes over a series of several APs. CaMKII phosphorylation alters inactivation and activation properties of LTCC current, moving the availability of Ca\(^{2+}\) channels to more positive membrane potentials and the activation to more negative potentials. In this way, CaMKII increases the window current (shaded field between the curves) of the \( I_{\text{Ca}} \), increasing the probability of Ca\(^{2+}\) channel openings and CaMKII activity by increasing Ca\(^{2+}\) entry through \( I_{\text{Ca}} \) and activating CaMKII (Figure 7). Facilitation increases sarcoplasmic (SR) Ca\(^{2+}\) leak and Ca\(^{2+}\)\(_{\text{i}}\), which in turn fuels the Na\(^{+}\)/Ca\(^{2+}\) exchanger (NCX) forward mode, causing delayed afterdepolarizations (DADs). Importantly, CaMKII integrates several proarhythmogenic mechanisms that may work to further augment CaMKII activity by increasing Ca\(^{2+}\)\(_{\text{i}}\) and ROS.

The proarrhythmic actions of CaMKII on CaV1.2 occur preferentially during the plateau phase of the cardiac action potential, at least in part because the plateau potential is an electric substrate that favors opening of CaV1.2, a "voltage-gated" ion channel in which plateau membrane potentials favor structural rearrangements of the channel protein that increase the probability of opening. Phosphorylation by CaMKII or by protein kinase A, the principal kinase activated by \( \beta \)-adrenergic receptor agonists, synergizes with cell membrane potential to enhance the probability of CaV1.2 opening. The relatively long cardiac action potential plateau in ventricular myocytes is necessary for myocardial physiology. The action potential plateau grades CaV1.2 openings that increase \( I_{\text{Ca}} \) and trigger SR Ca\(^{2+}\) release, which ultimately induces myofilament cross-bridge formation and cardiac systole, by a Ca\(^{2+}\)-induced Ca\(^{2+}\) release process (Figure 1). Unfortunately, the physiological advantages of the prolonged action potential plateau appear to go hand in glove with proarrhythmic vulnerability. Pathological action potential prolongation is a prominent outcome of a proarrhythmic electric remodeling process in heart failure in response to drugs and in the much rarer long-QT genetic arrhythmia syndromes (discussed below).

The Timothy syndrome is a very rare form of the long-QT syndrome caused by a mutation in CaV1.2 that prevents normal inactivation, leading to increased \( I_{\text{Ca}} \) action potential and QT interval prolongation, life-threatening arrhythmias, and multisystem defects. We found that CaMKII activation was essential for amplifying the genetic CaV1.2 defect in adult rat ventricular myocytes and that CaMKII inhibition normalized the action potential duration and eliminated afterdepolarizations, suggesting that CaMKII activation is part of a proarrhythmic feed-forward mechanism in at least 1 type of long-QT syndrome. Excessive action potential prolongation can promote arrhythmias by multiple mechanisms. CaV1.2 channels close and reopen within the action potential plateau, operating within a "window" between membrane potential–driven channel opening and inactivation (Figure 6). Ca\(^{2+}\) channel agonist drugs and CaMKII\(_{\text{II}}\) favor increased \( I_{\text{Ca}} \) window current during the action potential plateau. The membrane resistance is relatively high during the action potential pla-
According to the Ohm law (voltage equals current times resistance), small increases in inward current cause large depolarizations in membrane potential (voltage), which are the basis for EADs. The efficacy of Ca\textsuperscript{2+}/H\textsuperscript{1+} channel blockers as antiarrhythmic agents is limited, likely because concentrations adequate for significantly inhibiting I\textsubscript{Ca} prevent physiological cardiac and smooth muscle function, leading to inadequate inotropy and hypotension. Dihydropyridine CaV\textsubscript{1.2} antagonists work by preventing mode 2 gating\textsuperscript{58} and are effective at suppressing EADs in experimental animal models.\textsuperscript{62,65} In contrast, CaMKII\textsuperscript{54,57} and the CaV\textsubscript{1.2} channel agonist BayK \textsubscript{8644} increase CaV\textsubscript{1.2} mode 2 activity, I\textsubscript{Ca} window current, and EADs.\textsuperscript{56,71} CaMKII inhibition with a small-molecule CaMKII inhibitor (KN-93)\textsuperscript{54,74} or a calmodulin antagonist drug (W-7)\textsuperscript{63,75} is effective in preventing ventricular tachycardia. BayK \textsubscript{8644} induces mode 2 CaV\textsubscript{1.2} openings by direct actions on the pore-forming \(\alpha\) subunit that overrides the effects of CaMKII inhibition to reduce CaV\textsubscript{1.2} opening probability. The addition of BayK \textsubscript{8644} induces ventricular tachycardia even after treatment with the calmodulin antagonist W-7,\textsuperscript{63} a condition with reduced CaMKII activity, suggesting that I\textsubscript{Ca} is a critical site for proarrhythmic actions of CaMKII, a hypothesis supported by modeling studies.\textsuperscript{54,76} In our view, the best available evidence is that the proarrhythmic actions of CaMKII at CaV\textsubscript{1.2} occur by enhanced phosphorylation of a specific site (Thr498) on the CaV\textsubscript{1.2} \(\beta\) subunit protein because elimination of this site prevents mode 2 gating and significantly reduces EADs.\textsuperscript{55} It is interesting that the Thr498 site also contributes to intracellular Ca\textsuperscript{2+} overload, which contributes to cell death, suggesting the possibility that CaMKII proarrhythmic effects on CaV\textsubscript{1.2} may extend beyond arrhythmia triggering afterdepolarizations and include cell death, a feature of pathological tissue remodeling that contributes to SND\textsuperscript{30} and reentrant circuits (Figure 1).\textsuperscript{29}

CaMKII reduces the cell membrane voltage dependence of Na\textsuperscript{+} current (I\textsubscript{Na}) availability and at the same time increases a noninactivating component of I\textsubscript{Na} implicated in action potential prolongation and EADs by effects on the predominant cardiac voltage-gated Na\textsuperscript{+} channel, Na\textsubscript{1.5}.\textsuperscript{77} The reduction in Na\textsubscript{1.5} channels available to open resembles the Brugada syndrome,\textsuperscript{78} and the increase in the noninactivating component of I\textsubscript{Na} mirrors changes in I\textsubscript{Na} seen in heart failure,\textsuperscript{79} long-QT syndrome 3, and the presence of increased oxidant stress,\textsuperscript{80} suggesting the hypothesis that CaMKII is a common proarrhythmic signal for Na\textsubscript{1.5}. In cardiomyocytes and neurons, CaMKII is brought into close proximity to the Na\textsubscript{1.5} protein complex by \(\alpha\) spectrin, a cytoskeletal protein. Mutant mice lacking a CaMKII binding motif on \(\alpha\) spectrin are resistant to the proarrhythmic effects of CaMKII on I\textsubscript{Na} and EADs.\textsuperscript{81} Na\textsubscript{1.5} and CaV\textsubscript{1.2} share a general structure with 4 homologous “repeats” that comprise 6 transmembrane helixes (Figure 8). The 4-homologous-repeat domains are connected by intracellular linkers, and the linker domain between repeat I and II appears to be a “hot spot” for CaMKII-mediated phosphorylation and binding.\textsuperscript{81,82} Ser571 appears to be a key residue for phosphorylation in cardiomyocytes\textsuperscript{81} but not in HEK293 cells,\textsuperscript{82} in which Ser516 and Thr594 appear to be better CaMKII targets.
The complete effects of CaMKII on $I_{Na}$, reduced availability, and increased noninactivating late current are evident in cardiomyocytes but not in HEK293 cells, suggesting that multiple components contribute to the proarrhythmic actions of CaMKII on Na$_v$1.5. Both CaMKII inhibition and ranolazine, a drug that preferentially inhibits the noninactivating late component of $I_{Na}$, can reduce EADs, suggesting that Na$_v$1.5 late current is an important ion channel target for the proarrhythmic effects of CaMKII.

The Best Antiarrhythmic Drugs Are Not Ion Channel Antagonists

In contrast to the lack of success with ion channel antagonist drugs, antagonist drugs targeting neurohumoral pathways activated during conditions favoring common arrhythmias such as hypertension and myocardial infarction were found to be effective in reducing arrhythmias and sudden death. The β-adrenergic receptor antagonist drugs and drugs that inhibit the renin–angiotensin II–aldosterone system are effective for reducing heart failure and sudden death in patients with reduced left ventricular ejection fractions after myocardial infarction. Although these drugs engage specific receptors, they have widespread biological actions by virtue of the multivalent nature of signaling pathways activated by β-adrenergic, angiotensin, and mineralocorticoid receptors. Agonist stimulation at each of these signaling pathways can produce broad-based proarrhythmic and cardiomyopathic effects, which are associated with increased ROS and disturbed intracellular Ca$^{2+}$ homeostasis. The lack of efficacy of selectively targeted ion channel antagonist drugs, combined with the relative efficacy of neurohumoral antagonist drugs, suggests that successful antiarrhythmic therapies will need to address complex relationships between signaling pathways, myocardial hypertrophy, survival, and ion channels.

The Purpose of Ion Channels Is to “Trigger” Automaticity and Contraction

The purpose of the elaborate cell membrane (sarcolemmal) ultrastructure of cardiomyocytes and the rich variety of ion channel complexes is to initiate the coordinated contraction that is essential for physiologically tunable cardiac output. Phase 0 of the cardiac action potential is the “excitation” in excitation-contraction coupling. Cell membrane excitation (by $I_{Na}$ in the vast majority of cardiomyocytes) is coupled to mechanical duties and by $I_{Ca}$ in the small number of pacemaker and atrioventricular nodal cardiomyocytes) couples to contraction by a Ca$^{2+}$-induced Ca$^{2+}$ release process. In sinoatrial nodal pacemaker cells, which lack a clear mechanical purpose, excitation-contraction coupling is a misnomer. An arguably better descriptor for these pacemaker cells is excitation-excitation coupling because diastolic depolarization rate is a consequence of cell membrane excitation from $I_f$ and spontaneous SR Ca$^{2+}$ release that promotes $I_{NCX}$. In contracting cardiomyocytes, phase 0 depolarizes (ie, makes the inner cell membrane relatively more positive) the cell membrane, which enhances the opening probability of voltage-gated Ca$^{2+}$ channels (Figure 5). $I_{Ca}$ triggers a coherent release of relatively massive amounts of SR Ca$^{2+}$ from ryanodine receptors (Figure 1). The SR is the primary source of activator Ca$^{2+}$ for driving myofilament cross-bridge formation, which is the molecular basis for myocardial contraction. The peak $I_{Ca}$ represents the maximum number of open Ca$^{2+}$ channels in response to membrane depolarization. Soon after voltage-gated Ca$^{2+}$ channels open, they begin to inactivate and to become reluctant to reopen until completion of action potential repolarization (phase 3) and attainment of the physiologically negative resting cell membrane potential (phase 4). Diastole is an active process that requires reduction of myofilament-bound and free cytoplasmic Ca$^{2+}$ by active reuptake into the SR by the sarcoplasmic-endoplasmic reticulum ATPase (SERCA2a) and, to a lesser extent, by mitochondrial uptake. Cytoplasmic Ca$^{2+}$ removal also occurs by extrusion from the cell, mostly by the Na$^+$/Ca$^{2+}$ exchanger and to a lesser extent by the sarcolemmal Ca$^{2+}$ ATPase. Thus, the ion channel components that orchestrate myocardial cell excitability are intimately involved with cellular proteins required for intracellular Ca$^{2+}$ homeostasis and electric automaticity that underlies physiological pacing and mechanical control of myocardium. This interdependence of electric and Ca$^{2+}$ homeostatic systems occurs on an ultrastructure of cellular and organelle membranes that is best understood in ventricular myocytes (Figure 1). More than half of the ventricular myocyte membrane is involved in repetitively spaced invaginations called T tubules that reach deep into the myocyte interior. T-tubular membranes are richly decorated with voltage-gated Ca$^{2+}$ channels that face off across a narrow span of cytoplasm (∼10 nm) to engage dyadically arrayed ryanodine receptors. These dyads provide a nearly ideal spatial environment for Ca$^{2+}$-induced Ca$^{2+}$ release. However, when these relationships are disturbed at an ultrastructural and a molecular level, as occurs in myocardial injury, they become prone to arrhythmias and sudden death.

Managing intracellular Ca$^{2+}$ constitutes a major ATP cost for cardiomyocytes. The Ca$^{2+}$ concentration gradient between the extracellular space (∼1 mmol/L) and cytoplasm (∼100 mmol/L) is massive (∼10 000 times more extracellular than bulk cytoplasmic Ca$^{2+}$). There is broad variation in the concentration of free versus protein-bound intracellular Ca$^{2+}$, spatially and over time (eg, systole versus diastole), so intracellular Ca$^{2+}$ in the SR and in the dyadic space between voltage-gated Ca$^{2+}$ channels, integral to T-tubular membranes, and SR-bound ryanodine receptors during systole may approach extracellular values. CaMKII catalyzes the phosphorylation of important Ca$^{2+}$ homeostatic proteins, including voltage-gated Ca$^{2+}$ channels,54,56,57 ryanodine receptors,57–59 and the SERCA2a regulatory protein lamban.90 Thus, CaMKII is positioned to enhance cellular Ca$^{2+}$ fluxes and to coordinate physiological goals of excitation-contraction coupling, lusitropy, and heart rate. It is therefore not necessarily surprising that, when excessively activated, CaMKII may also contribute to pathological rearrangement of membrane excitability and mechanical function, promoting arrhythmias and heart failure.

Understanding Non–Ion Channel Proarrhythmic Actions of CaMKII

Although ion channels are the final effectors of cell membrane excitability, multiple cellular and tissue events contrib-
ute to arrhythmia initiation and perpetuation. The proarrhythmic effects of excessive CaMKII activity are due to actions at multiple protein targets that affect intracellular Ca$^{2+}$ homeostasis, myocardial survival, matrix, and inflammation.

**Intracellular Ca$^{2+}$ Homeostasis**

CaMKII has been shown to regulate both SR Ca$^{2+}$ uptake and release. For relaxation to occur, the largest fraction of the cytosolic Ca$^{2+}$ is removed to the SR by SERCA2a, which is inhibited by phospholamban. CaMKII phosphorylates phospholamban at Thr17,90 which reduces the inhibitory effect of phospholamban on SERCA2a, thereby increasing Ca$^{2+}$ reuptake by the SR and myocardial relaxation. CaMKII catalyzes phosphorylation of several known sites on the cardiac ryanodine receptor.87,88 One highly investigated site is Ser2814.89 CaMKII-dependent phosphorylation of Ser2814 increases proarrhythmic diastolic SR Ca leak.24 Diastolic SR Ca$^{2+}$ leak (measured as Ca$^{2+}$ sparks91 and waves) increases cytosolic Ca$^{2+}$ and reduces SR Ca$^{2+}$ content, which in turn increases forward-mode $I_{SCX}$, leading to (late phase 3) EADs and DADs. There is a growing body of evidence that this concept of CaMKII- and afterdepolarization-dependent arrhythmogenesis is valid for both ventricular and atrial tachyarrhythmias, including AF (discussed below). Increased diastolic SR Ca$^{2+}$ leak and hyperphosphorylation of ryanodine receptors are also features of heart failure,92 which may contribute to impaired contractility and arrhythmias.93

**Cell Death**

Excessive CaMKII activity induces cell death, which can contribute to reparative fibrosis and adverse remodeling.20,30,32,94 In noncardiac tissues, CaMKII$\gamma$ promotes apoptosis by Fas death receptor and mitochondrial pathways.95 In cardiac tissue, excessive and sustained $\beta_1$-adrenergic receptor stimulation results in increased apoptosis, adverse remodeling, and heart failure. CaMKII is critically involved in $\beta_1$-adrenergic receptor–mediated apoptosis, and mice with transgenic expression of the CaMKII-inhibitory protein AC3-I are in part protected from apoptosis on $\beta_1$-adrenergic receptor stimulation and after myocardial infarction.94,96

**Inflammation**

Inflammation is a feature of arrhythmia-prone myocardium after myocardial infarction, in various cardiomyopathies, and in AF.97–99 CaMKII is involved in the induction of the sarcoclemmal injury by activating a local inflammatory response through the nuclear factor-κB pathway.99 CaMKII is also oxidatively activated during myocardial infarction or by endotoxin as part of a Toll-like receptor/MyD88 pathway.100 The potential for CaMKII to activate and to be activated by inflammatory signaling suggests that CaMKII may be an important molecular connection between inflammation and arrhythmias.

**Extracellular Matrix and Scar Formation**

We recently found that aldosterone leads to oxidative activation of CaMKII and myocardial matrix metalloproteinase-9 synthesis by activation of a myocyte enhancer factor 2 transcriptional pathway.35 In the setting of myocardial infarction and hyperaldosteronism, myocardial matrix metalloproteinase-9 production was sufficient to increase the likelihood of death caused by myocardial rupture. The untoward effects of myocardial CaMKII on extracellular matrix are potentially consistent with the antifibrotic effects of myocardial CaMKII inhibition,90 a contributing factor in SND and reentrant arrhythmias. Oxidized CaMKII may contribute to peri-infarct scar properties that promote reentrant arrhythmias.29 CaMKII can also increase fibrosis as a consequence of promoting cell death. CaMKII actions at Ca$^{2+}$ homeostatic proteins14 and mitochondria55 appear to activate myocyte death programs under conditions of disease stress. Thus, myocardial CaMKII has the potential to modify tissue substrates that can promote arrhythmias by contributing to structural heart disease.

**Overview of Arrhythmias Linked to CaMKII**

**Atrial Fibrillation**

A growing body of evidence suggests an important causative role for CaMKII in AF. The hallmarks of abnormal cell membrane excitability in AF are enhanced triggered excitability and automaticity and reduced refractoriness owing to shortened action potential duration. Profound fibrosis and atrial dilatation of the fibrillating atria provide the tissue substrate for conduction slowing, reentrant circuits, and maintenance of AF. About a decade ago, Tessier et al.101 discovered that CaMKII$\delta$ expression is increased in chronic human fibrillating atria. They provided evidence, corroborated by others,102 that CaMKII enhances the repolarizing transient outward K$^+$ current ($I_{to}$) in fibrillating human atrial cardiomyocytes, thus shortening refractoriness and action potential duration, and that CaMKII leads to a disturbed frequency-dependent reactivation of $I_{to}$. Both mechanisms are proarrhythmogenic per se, favoring functional reentry circuits and dispersion of atrial excitability. Indeed, direct CaMKII inhibition (by KN-93 and autacamide–2–related inhibitory peptide) and indirect inhibition (by the calmodulin inhibitor calmidazolium) significantly reduced these effects. Enhanced CaMKII activity in fibrillating atria in animal models103 and patients104 causes hyperphosphorylation of the ryanodine receptor at Ser2814, leading to increased diastolic SR Ca$^{2+}$ leak,104,105 elevated cytosolic Ca$^{2+}$, and increased susceptibility to AF.103,104 This mechanism has been recently validated in human fibrillating atrial myocytes, tying CaMKII-dependent diastolic SR Ca$^{2+}$ leak to the increased frequency of DADs as a trigger for AF.106 Consistently, CaMKII inhibition blocked this AF-triggering pathway at all levels by reducing SR Ca$^{2+}$ leak,103,104 DADs, ectopic beats,103,106 and inducible AF.103

**Sinus Node Dysfunction**

SND results from defective impulse formation or propagation in the sinoatrial node or adjacent atrial myocardium. Patients with SND present with a variety of rhythm abnormalities, including sinus bradycardia, sinus pauses and arrest, intermittent exit block, and deficient heart rate response to exertion or chronotropic incompetence. In contrast to extrinsic and reversible SND (eg, resulting from hypoxia, metabolic disturbance, or drugs), intrinsic SND is not readily reversed.
because of pacemaker cell death and replacement fibrosis. The loss of sinoatrial node cells leads to physiologically inadequate summation and formation of the propagating pacemaker impulse, a phenomenon also referred to as electric “source-sink mismatch.” Typically, intrinsic SND occurs in conditions of increased oxidative stress and high amounts of circulating angiotensin II, as occurs in elderly patients with hypertension, structural heart disease, and heart failure. We recently reported a model of SND in mice induced by long-term angiotensin II treatment. These mice had sinus pauses and chronotropic incompetence, and their sinoatrial node regions showed cell loss resulting from increased cell death with extensive fibrosis. In our model, angiotensin II induced ROS through a NADPH oxidase–dependent pathway, and ROS activated CaMKII (oxidized CaMKII), and we found that oxidized CaMKII was elevated in right atria in patients who required a pacemaker for SND. Sinoatrial node gene painting with focal pacemaker expression of a synthetic CaMKII inhibitory peptide protected angiotensin II–infused mice from SND and pacemaker cell death by preventing a source-sink mismatch between the sinus node and the surrounding atrium. These data suggest that SND could be prevented in high-risk patients by CaMKII inhibition targeted to sinoatrial nodal pacemaker cells.

**Ventricular Tachyarrhythmias in Structural Heart Disease**

Ventricular tachycardia and ventricular fibrillation are common causes of sudden death, as discussed earlier. Patients with structural heart disease are at highest risk, and myocardium from patients and from animal models of structural heart disease shows a consistent increase in the expression and/or activity of CaMKII. There is now abundant evidence that CaMKII overactivity can promote potentially lethal ventricular arrhythmias by initiating afterdepolarizations and causing proarrhythmic tissue remodeling that favors reentry. Furthermore, CaMKII inhibition by small molecules and genetic approaches is effective in preventing or reducing ventricular arrhythmias in animal models in vivo and isolated tissues and ventricular myocytes from animals and in reducing proarrhythmic SR Ca$^{2+}$ leak and improving contractility in failing human ventricles. These data, from diverse sources, support the view that CaMKII can contribute to the initiation and perpetuation of ventricular arrhythmias and that CaMKII inhibition is an effective antiarrhythmic therapy.

**Inherited Tachyarrhythmias**

CaMKII has been implicated in a number of genetic arrhythmia syndromes, including the Brugada syndrome, long-QT syndromes, particularly long-QT syndrome 3 and the Timothy syndrome, and catecholaminergic polymorphic ventricular tachycardia. CaMKII may be part of a feed-forward proarrhythmic circuit in each of these examples by virtue of the tendency of CaMKII to become constitutively active under conditions of increased Ca$^{2+}$ (eg, catecholaminergic polymorphic ventricular tachycardia in which ryanodine receptors are “leaky”), prolonged intracellular Ca$^{2+}$ transients (eg, action potential prolongation in the long-QT syndromes), or rapidly repetitive Ca$^{2+}$, as occurs in all tachyarrhythmias. CaMKII inhibition has been effective in reducing arrhythmias in animal models of genetic arrhythmias, suggesting that CaMKII inhibitory therapy could be useful in reducing the risk of arrhythmia in patients.

**Conclusions**

A modern understanding of arrhythmia mechanisms acknowledges that proarrhythmic behaviors of ion channels depend on a cellular and tissue context, which together contribute to common clinical arrhythmias. It is increasingly clear that CaMKII regulates physiological connections between ion channels and intracellular Ca$^{2+}$ homeostasis in myocardium. Under pathological acquired or genetic stress, excessive CaMKII activity promotes bradyarrhythmias and tachyarrhythmias. Proof-of-concept studies in animal models consistently show that CaMKII inhibition provides antiarrhythmic benefits. Thus, our field awaits the development of clinically applicable CaMKII inhibitory drugs to determine whether the experimentally observed benefits of CaMKII inhibition will also improve the lives of patients. Many questions remain, including whether systemic CaMKII will be tolerated, and multiple steps will be necessary, including the development of small molecules with drug-like properties and adequate specificity, features that are lacking in currently available experimental inhibitors, before the clinical efficacy of CaMKII inhibition for treating arrhythmias can be tested.

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**Disclosures**

Dr Anderson is a named inventor on intellectual property claiming to treat arrhythmias by CaMKII inhibition and is a cofounder of Allosteros Therapeutics, a biotech company aiming to develop enzyme-based therapies. Dr Rokita reports no conflicts.

**References**


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