Sudden cardiac death (SCD) refers to a sudden unexpected death from cardiac causes and is a major cause of death worldwide.\(^1\) In patients with structural heart disease, arrhythmias are the main cause of death.\(^2\) Despite improvements in primary and secondary prevention with substantial decline in mortality of coronary heart disease in recent years,\(^{1,3}\) SCD rates have declined to a much lesser extent.\(^4\) Implantable cardioverter-defibrillators can reduce cardiovascular mortality in high risk patients, but prevention of SCD is particularly challenging because the majority of cases occur in individuals

**Abstract:** Despite improvements in the therapy of underlying heart disease, sudden cardiac death is a major cause of death worldwide. Disturbed Na and Ca handling is known to be a major predisposing factor for life-threatening tachyarrhythmias. In cardiomyocytes, many ion channels and transporters, including voltage-gated Na and Ca channels, cardiac ryanodine receptors, Na/Ca-exchanger, and SR Ca-ATPase are involved in this regulation. We have learned a lot about the pathophysiological relevance of disturbed ion channel function from monogenetic disorders. Changes in the gating of a single ion channel and the activity of an ion pump suffice to dramatically increase the propensity for arrhythmias even in structurally normal hearts. Nevertheless, patients with heart failure with acquired dysfunction in many ion channels and transporters exhibit profound dysregulation of Na and Ca handling and Ca/calmodulin-dependent protein kinase and are especially prone to arrhythmias. A deeper understanding of the underlying arrhythmic principles is mandatory if we are to improve their outcome. This review addresses basic tachyarrhythmic mechanisms, the underlying ionic mechanisms and the consequences for ion homeostasis, and the situation in complex diseases like heart failure. (Circ Res. 2015;116:1956-1970. DOI: 10.1161/CIRCRESAHA.116.304678.)

**Key Words:** alternans ■ arrhythmias ■ calcium ■ calcium/calmodulin-dependent kinase II ■ sodium
Excitation–Contraction Coupling

To understand arrhythmogenesis, basic knowledge about the fundamental principles of excitation–contraction coupling is required (Figure 1). Cardiac excitation is based on sarclemmal ion fluxes that are tightly coupled to cytosolic Ca and contraction.7 Excitation is initiated by opening of voltage-gated Na channels and Na current influx (I_{Na}), leading to the rapid action potential (AP) upstroke (phase 0).29 Phase 0 is limited by fast I_{Na} inactivation and the activation of transient outward K channels (K_{Ca}), contributing as the membrane repolarizes. K_{Ca} also stabilizes the plateau membrane potential, resulting in arrhythmias. I_{Ca} (which activates more slowly than I_{Na}) also progressively decays during the plateau. In ventricular cells, phase 3 repolarization ensues as K currents slowly activate (I_{Ks}, carried by Kv7.1, KCNQ1) or undergo voltage-dependent recovery from inactivation (I_{Kr}, carried by hERG, KCNH2) and I_{K1} (carried by Kir2.x channels) progressively contributes as the membrane repolarizes. I_{K1} also stabilizes diastolic V_{m} near ~80 mV during phase 4. In pacemaker cells, the relative paucity of stabilizing I_{Kr}, together with the prominence of the hyperpolarization-activated nonspecific cation current I_{f} (HCN channels) and Ca release driven I_{Ca,NCX}, are mainly responsible for diastolic depolarization, leading to AP initiation and pacemaker function.7

The cardiomyocyte membrane (sarcolemmal) has transverse invaginations called T-tubules (≈200 nm diameter) that reach deep into the myocytes interior. T-tubules make close contact with enlarged junctional sarcoplasmic reticulum (SR)
cisternae, with only a 14 nm wide dyadic cleft that separates the SR and T-tubular membranes. L-type Ca channels are highly concentrated in the T-tubules at these junctional clefts and are in close proximity to cardiac ryanodine receptors (RyR2), the SR Ca release channel (Ca release microdomain). This L-type Ca channels-RyR2 proximity at the dyadic cleft means that Ca ions that enter via $I_{Ca}$ cause local cleft [Ca] to be high, sufficient to trigger the opening of RyR2 clusters responsible for SR Ca release during excitation–contraction coupling. This Ca-induced Ca release amplifies the sarcolemmal Ca influx, and depending on species and heart rate, the SR Ca release accounts for 70% to 92% of total Ca increase during systole. Total Ca is heavily buffered (≈100:1), but free cytosolic [Ca] typically increases from 100 to 600 nmol/L, which leads to myofilament activation and contraction (systole). The [Ca] rise is transient because $I_{Ca}$ inactivation and RyR2 closure limit cytosolic Ca entry, and at the same time, Ca removal pathways are activated. For Ca removal, the 2 major pathways are SR Ca-ATPase (SERCA2a) and sarcolemmal Na/Ca exchange, although a tiny fraction of Ca can be extruded by the sarcolemmal Ca-ATPase and mitochondrial Ca unipporter. During steady state, the proportion of Ca transported into SR and out of the cell correspond exactly to the amount of Ca released from the SR and sarcolemmal Ca influx, respectively.

CaMKII

CaMKII is a serine/threonine kinase that can be activated by Ca/calmodulin binding to its regulatory domain. This activation results in the phosphorylation of target proteins, including RyR2, Na$_v$1.5, L-type Ca channels, and phospholamban (the negative regulator of SERCA2a). Therefore, CaMKII is crucially involved in the regulation of excitation–contraction coupling.

CaMKII assembles into a dodecameric holoenzyme consisting of 2 stacked hexamers. One of the target proteins of CaMKII is the regulatory subunit of an adjacent CaMKII monomer. This auto-phosphorylation results in augmented activation that persists even after dissociation from Ca/CaM and provides an integrated response from the short-term fluctuations in intracellular Ca. It was also shown that reactive oxygen species (ROS)–dependent oxidation of CaMKII at 2 methionine residues at the regulatory domain (M281/282) results in autonomous activation similar to autophosphorylation. Thus, CaMKII can link increased ROS to their downstream effectors. Increased expression and activity of CaMKII has been linked to contractile dysfunction and arrhythmias in HF and on conditions of increased ROS production.

Mechanisms of Electric Instability

Ventricular tachycardia or fibrillation are the result of cell membrane hyperexcitability, disturbed repolarization, or defective conduction of the electric wavefront across the myocardium. The presence of an electrically inactive scar tissue that often develops after myocardial infarction forces the wavefront to propagate around this line of block. The wavefront then enters the area behind the scar. If that wavefront reaches the back of the obstacle from multiple directions simultaneously (or near the same time), then the waves will annihilate (Figure 2A). However, if unidirectional block (often coupled with decremental conduction) exists around one side, the wavefront can

Figure 2. Proarrhythmic mechanisms. A, Reentry. Upper panel, Normal conduction around an obstacle. The latter exhibits electric properties that differ dramatically from the rest of the myocardium (slowed conduction and repolarization). The wavefront propagates around this line of block and reaches the back from multiple directions simultaneously, resulting in wave annihilation. If, however, unidirectional block (often coupled with decremental conduction) exists around one side (lower panel), the wavefront can reenter and re-excite tissue in front of the unidirectional block. This can lead to stable reentry. B, Triggered activity. Increased depolarizing currents that result in prolongation of action potential duration (APD) can favor L-type Ca channel reactivation. Increased Ca entry via $I_{Ca}$ can also lead to sarcoplasmic reticulum (SR) Ca overload and spontaneous SR Ca release during AP plateau phase. This may generate transient inward ($I_{Ti}$) current by Na/Ca exchange. Both Ca channel reactivation and $I_{Ti}$ may depolarize the membrane during AP plateau phase, which can result in an early afterdepolarization (EAD). In addition, SR Ca overload may also result in diastolic SR Ca release. The consequent $I_{Ca}$ may lead to depolarization from the resting membrane potential, which can result in a delayed afterdepolarization (DAD). Partially derived from Bers.23
reenter and re-excite tissue in front of the unidirectional block (Figure 2A). This can lead to stable macroscopic re-entry. Reentry can also occur around other functional obstacles (e.g., depolarized tissue) and can take the form of a rotor.22,24 Stable rotors lead to monomorphic VT. If the waves that propagate outward from a rotor develop wavebreaks, additional rotors are generated, which may lead to polymorphic VT and VF.24 This development is much more likely under conditions of altered excitation, repolarization, or conduction because of disturbed ion channel function (Figure 3). A typical cause for this electric remodeling is structural remodeling associated with cardiac disease secondary to advanced age, increased oxidant stress, hypertension, diabetes mellitus, tissue injury, or inflammation.22 However, the disturbed function of ion channels that underlies these changes in excitation, repolarization, or conduction can also occur in structurally normal myocardium. Abnormal impulse conduction with consequent reentrant excitation can also be a consequence of Ca alternans (Figure 3). This is an example of how Ca dysregulation can cause reentry, which is the most common mechanism of electric instability.

Beside reentry, triggered activity is another important mechanism of electric instability. The latter is the result of an imbalance of ion currents within a single cardiomyocyte. Such imbalances can be classified as either early or delayed depending on their occurrence during the AP. Early afterdepolarizations (EADs) occur during the plateau phase of a cardiac AP, whereas delayed afterdepolarizations (DADs) disturb the diastolic membrane potential after AP repolarization is complete (Figure 2B). Both EADs and DADs can reach the threshold inducing premature ventricular contractions, which can degenerate into monomorphic or polymorphic VTs.

Early Afterdepolarization

EADs occur during the AP and are a consequence of increased inward currents or reduced repolarization reserve (reduced outward K currents).7 The plateau phase of the AP is especially vulnerable because repolarizing and depolarizing currents are small and nearly balanced. During this phase, small alterations in the amplitude of even one ionic current can result in the generation of an EADs. We have learned much about the fundamental mechanisms of triggered activity by studying familial disorders with prolonged repolarization. For example, congenital long QT syndrome 3 is characterized by profound AP prolongation because of increased persistent or late Na current (late I Na),25 EADs are a major trigger for tachyarrhythmias, but can also occur at low heart rates (where AP duration [APD] is intrinsically long). The long APD allows for reactivation of I Ca at plateau potentials. The resulting inward current creates an EAD as a depolarization in the otherwise monotonically decaying plateau voltage.26,27 Among the long QT syndromes, the Timothy syndrome is a rare form, caused by a mutation in Ca 1.2 that prevents normal I Ca inactivation. This disease is characterized by AP prolongation, increased QT intervals, life-threatening arrhythmias, and multisystem defects. Similar to other causes of AP prolongation, I Ca reactivation is a major cause of EAD formation. Interestingly, pathological AP prolongation is a prominent feature of a proarrhythmic electric remodeling in HF.28,29 but can also occur in response to drugs that inhibit K channels (especially I Ks). This highlights the importance of this fundamental arrhythmic mechanism.

Beside these well-studied EADs arising during long AP plateaus via I Ca reactivation, there is now compelling evidence that some EADs are initiated by SR Ca rerelease during the AP that causes inward I NCX. These EADs are more common during repolarization and are sometimes called phase 3 EADs. The inward I NCX may only cause a weak depolarization, but that can be amplified by reactivation of I Ca10,31 depending on the voltage. That is, in atrial (and rodent ventricular) APs with I NCX-prolonged late and plateau at voltages below −40mV (where I Ca reactivation does not occur), EADs may be driven by nonequilibrium reactivation of I NCX which is facilitated by increased SR Ca release.32

Delayed Afterdepolarization

DADs are a consequence of cytosolic and SR Ca overload.23 Both increased cytosolic and SR luminal Ca increase the diastolic open probability of cardiac RyR2 7 Ca sparks are elementary SR Ca release events that occur if a cluster of RyR2...
open. During systolic Ca-induced Ca release, Ca sparks are synchronized within the cell and summate to form the Ca transient. Spontaneous Ca sparks are mainly responsible for diastolic SR Ca release and diastolic SR Ca leak via RyR2. Because RyR2 is in relatively close proximity to the NCX, this localized [Ca]i elevation drives inward $I_{\text{NCX}}$. Note that the stoichiometry of Na/Ca exchange (3Na+:1Ca2+) meaning that Ca extrusion is accompanied by a net inward movement of one positive charge via $I_{\text{NCX}}$, which is almost entirely responsible for what used to be called transient inward current $I_{\text{cT}}$. This $I_{\text{NCX}}$ is large enough, it can cause and appreciate DAD, which can trigger an arrhythmic AP. Individual Ca sparks normally do not produce enough $I_{\text{NCX}}$ to produce a measurable DAD because they are isolated unsynchronized events. However, at higher SR Ca content, the Ca sparks are larger in amplitude and nearby RyR2 clusters are more sensitive to activation, resulting in a cell-wide Ca wave with sufficient $I_{\text{NCX}}$ to cause DADs and triggered APs. Sympathetic stimulation can drive up SR Ca content and increase the propensity for Ca waves and triggered beats.

In the intact heart, the $I_{\text{NCX}}$ produced by a Ca wave in a single myocyte is insufficient to trigger an appreciable DAD or AP because all of the neighboring cells can effectively clamp that single cell at the diastolic voltage. However, when these Ca waves and consequent $I_{\text{NCX}}$ are synchronized regionally among many cells (by the prior AP and similar RyR2 recovery kinetics), that region can initiate triggered beats as premature ventricular contractions for the whole heart. The heart is relatively protected from these triggered arrhythmias initiated via EADs and DADs, but under pathological conditions caused by either genetic ion channel/transporter mutations or acquired diseases like HF, both the cellular propensity for EADs and DADs and their ability to cause whole heart premature ventricular contractions can be greatly increased.

### Cardiac Alternans

Cardiac alternans is a known risk factor for cardiac arrhythmias and SCD. The transition from monomorphic VT to polymorphic VT or VF requires breaks in the wave propagating from a stable rotor. During VT, cardiomyocyte Ca cycles at high rates. The time for intracellular Ca removal is drastically shortened and can elevate diastolic [Ca], and limit SR Ca load. This can also encroach upon the recovery time of the $I_{\text{Ca}}$-induced SR Ca release. Normally, [Ca] is tightly controlled, but Ca dysregulation can predispose to afterdepolarizations and cardiac alternans. Cardiac alternans are alternating beats with large/small amplitude Ca transient and long/short APD (Figure 4A). Most common in large mammals are electromechanically (or $V_m$-Ca) concordant alternans in which the large Ca transient and contraction is associated with the longer APD. However, $V_m$-Ca discordant alternans can also occur. Alternans can also be spatially discordant, where all regions of the ventricle are in phase with each other, or spatially discordant where different regions are out of phase with each other. As we will see the Ca and $V_m$ changes are usually functionally linked, but both can occur independently.

### Repolarization Alternans

Alternation in APD is called repolarization alternans. Because this type of alternans involves membrane potential, it can be clinically observed as T-wave alternans (TWA). TWA has been shown to be associated with cardiac arrhythmias and SCD. TWA is not restricted to a specific underlying disease but can be observed in HF, Brugada syndrome, and long QT syndrome.

TWA can result from changes in sarcomembranual ion current recovery (typically of $I_{\text{Na}}$ or $I_{\text{Ca}}$) manifested by a steep slope of cellular APD restitution. This type of TWA typically occurs at high heart rates with reduced diastolic intervals. The dependence of APD and conduction velocity (CV) on the preceding diastolic interval are called APD restitution and CV restitution, respectively. Shorter diastolic intervals result in shorter APD and slower CV. Both APD and CV restitution critically depend on the speed of recovery from inactivation of sarcomembranual ion channels. Nevertheless, other mechanisms, for instance SR Ca release, can also contribute to APD restitution changes. Intracellular Ca released from the SR inactivates $I_{\text{Ca}}$. A large SR Ca release could, therefore, result in a more pronounced Ca-dependent inactivation of $I_{\text{Ca}}$, which would influence APD restitution.

CV restitution, on the other hand, mainly depends on Na channel recovery. This is because CV depends on AP upstroke velocity, and the latter is determined by the magnitude of $I_{\text{Na}}$. Na channel recovery is usually fast; thus, CV restitution occurs only at high heart rates, as would be the influence of Na channel recovery on APD restitution. However, under conditions of slowed Na channel recovery, the impact on CV restitution (and also APD restitution) may already occur at much lower heart rates. This is important because slowed Na channel recovery has been shown to be a feature of many cardiac diseases like HF or ischemia (where CaMKII may be involved) and Brugada syndrome.

As mentioned above, APD restitution depends on sarcomembranual ion channel recovery. Among them, L-type Ca channels are very important. $I_{\text{Ca}}$ is the major inward current during the AP plateau. As stated earlier, AP plateau is the most vulnerable phase of the AP. This explains why inhibition of $I_{\text{Ca}}$ has been shown to reduce the slope of the APD restitution curve. In addition, the recovery of Ca channels is slower compared with, for instance, Na channels. Therefore, Ca channels influence APD restitution already at lower heart rates. Interestingly, because $I_{\text{Ca}}$ also determines SR Ca load, and vice versa, APD alternans and Ca alternans are functionally linked.

Repolarization alternans can be either spatially concordant or discordant. It was shown that Na channel recovery is critically involved in spatially discordant alternans. Because spatially discordant alternans is accompanied by changes in CV, both T wave and QRS are affected, resulting in T-wave and QRS alternans (Figure 6). Repolarization alternans can be proarrhythmic by 2 mechanisms. First, spatially discordant alternans results in spatial dispersion of repolarization providing a substrate for reentry. Second, at high heart rates, alternans can promote conduction block, which may trigger rotor formation. Alternans-dependent arrhythmias are, therefore, most likely generated under conditions of increased...
heart rate like during exercise or occur secondary to a stable rotor-dependent ventricular tachycardia. In fact, alternans may mechanistically explain why a stable ventricular tachycardia may degenerate into a multiple wavelet-driven polymorphic ventricular tachycardia or VF. Importantly, if APD is substantially prolonged, which is a typical feature of HF or long QT syndrome, ion channel recovery is already impaired at lower heart rates. Even without AP prolongation, if Na channel recovery is impaired like in HF or ischemia, and Brugada syndrome, alternans-dependent arrhythmias can occur at much lower heart rates.

**Ca Alternans**

From the surface electric measurements of an ECG, it is impossible to state whether APD alternans is accompanied by Ca alternans or whether one leads to the other. However, extensive work over the past years has shown that Ca and APD alternans usually coexist, are mechanistically linked, and that...
Ca-driven alternans seems to be more clinically relevant in the setting of heart disease.\textsuperscript{56}

Alternans start to occur as pacing rate is increased, and Ca alternans occur even when myocytes are voltage clamped with identical $V_m$ waveforms, consistent with Ca-driven alternans.\textsuperscript{47,48,67}

In the absence of voltage clamp, those Ca alternans also cause APD alternans, and there are logical reasons why a larger versus smaller Ca transient would alter APD. The 2 most prominent [Ca]\textsubscript{I}-dependent currents are $I_{Ca}$ and $I_{NCX}$. A large Ca transient would (a) strengthen Ca-dependent inactivation of $I_{Ca}$, which would shorten APD, and (b) drive increased inward $I_{NCX}$, which would prolong APD. In mammals with prominent AP plateaus, concordant $V_m$-Ca alternans usually predominate, suggesting that the $I_{NCX}$ effects are more powerful contributors to alternans. However, some conditions could shift this balance to favor a predominant role for $I_{Ca}$ inactivation and discordant $V_m$-Ca alternans. As stimulation rate increases, alternans start at a certain threshold and the alternans ratio (large:small Ca transient amplitude or APD) increases progressively. This can be seen as alternans in the amount of SR Ca release in direct measurements of intra-SR free [Ca] ($[Ca]_{SR}$), in both isolated myocytes and whole heart (Figure 4A).\textsuperscript{43,44}

Several factors contribute to alternans, and they seem to follow a sequence. The first step seems to be an encroachment on RyR2 recovery so that SR Ca release decreases, despite unaltered APD, $I_{Ca}$, and $[Ca]_{SR}$.\textsuperscript{43,44,68}

This is because RyR2 restitution is much slower than normal $I_{Ca}$ restitution. The partial failure of SR Ca release at this first small beat allows improved RyR2 recovery to occur at the next large beat, but then the cycle repeats. SR Ca release alternans can appear at heart rates where the consequent APD alternans are not yet detectable (Figure 4B). As Ca release alternans grow, they are amplified by alternating changes in SR Ca load, that is, SR Ca load alternans (Figure 4C). The small release limits Ca-dependent inactivation of $I_{Ca}$, thereby increasing Ca influx and load for the next (large) beat. The small Ca transient also drives less Ca extrusion via NCX, which limits Ca loss at the small beat. Then at the large beat, there is greater Ca-dependent $I_{Ca}$ inactivation and greater Ca efflux via NCX, which reduces cell and SR Ca load, setting the scene for stable alternans, \( \leq 6 \) to 7 Hz (150 ms cycle length) in rabbit hearts (Figure 4C). The steep nonlinear dependence of SR Ca release on diastolic SR Ca load\textsuperscript{42,49} enhances the impact of the SR load alternans on Ca release alternans.\textsuperscript{59} At even higher stimulation rates, shorter diastolic intervals or more depolarized diastolic voltage, one can encroach on $I_{Na}$ (or even $I_{Ca}$) restitution, which may also exacerbate a sort of Ca-driven alternans (smaller $I_{Ca}$ causes smaller Ca transient and APD). But here the lines become blurred with the electric, restitution-driven alternans involving APD and CV restitution, as discussed above.

Several factors can influence the pacing rate at which alternans is observed. Inhibition of glycolysis, mitochondrial energetic limitations, decreased SERCA function, and redox modification of RyR2 can all favor alternans.\textsuperscript{70–73} All of these factors may be relevant in pathophysiological conditions like HF, thus enhancing the propensity for alternans and arrhythmogenic sequelae in these conditions.

Similar to repolarization alternans, Ca alternans can be spatially in phase (concordant) or out of phase, that is, discordant, in different regions of a single myocytes\textsuperscript{74–76} or hearts, and spatially discordant alternans have been associated with lethal VTs and VF in patients.\textsuperscript{51,77}

### Arrhythmias in HF and Ischemia/Reperfusion

Despite improvements in HF therapy and ischemia/reperfusion associated myocardial damage, it has proven difficult to develop antiarrhythmic treatments and prevent SCD. Conventional ion channel blockers that are used as antiarrhythmic drugs are known to induce arrhythmias in structural heart disease.

There is an increasing body of evidence that HF and ischemic heart disease are accompanied by alternations in intracellular Na and Ca handling. Both conditions are characterized by an increased generation of ROS,\textsuperscript{78–80} which may contribute to disturbed Na and Ca handling.\textsuperscript{81} Changes in intracellular Na and Ca handling are associated with electric instability, and many of these alterations are linked to both contractile dysfunction and arrhythmias.

#### Late Na Current

As stated earlier, APD is increased in HF.\textsuperscript{29} Beside reduction of K current expression, increased magnitude of late $I_{Na}$ has been shown to contribute to AP prolongation in HF\textsuperscript{52} and after myocardial infarction.\textsuperscript{83} Late $I_{Na}$ is generated by dysfunctional inactivation of cardiac voltage-gated Na channels Na\textsubscript{1,5}.\textsuperscript{84} The detailed molecular mechanism is not fully understood.\textsuperscript{85}

Although late $I_{Na}$ has a small amplitude compared with peak $I_{Na,AP}$, it persists for hundreds of milliseconds during the cardiac AP,\textsuperscript{86} providing a source for increasing [Na].\textsuperscript{51,16} Increased [Na] is a well-known feature of HF, ischemia/reperfusion, or other conditions of increased ROS production and can contribute to contractile dysfunction and arrhythmias.\textsuperscript{16,19,25,87–91}

The late $I_{Na}$-dependent prolongation of the AP plateau renders the membrane potential vulnerable for EADs (Figure 5).\textsuperscript{16} A similar arrhythmogenic mechanism can be found in familial long QT syndrome 3. Mutations in the gene encoding Na\textsubscript{1,5} (SCN5A) have been shown to increase late $I_{Na}$, leading to AP prolongation and EADs.\textsuperscript{92,93} Transmural differences in late $I_{Na}$ might also increase dispersion of repolarization,\textsuperscript{94} which underlies the development of torsade de pointes tachyarrhythmias. In addition, late $I_{Na}$ is involved in cardiac alternans: ischemia-induced spatially discordant repolarization alternans has been shown to be prevented by inhibition of late $I_{Na}$.\textsuperscript{95}

Beside AP prolongation, late $I_{Na}$-dependent Na overload leads to intracellular Ca accumulation either by reduced Ca exit because of limitations to Ca extrusion by NCX or by additional Ca entry via reverse mode NCX.\textsuperscript{16} Intracellular Ca accumulation is associated with increased diastolic SR Ca leak and DADs (Figure 5). Moreover, it contributes to diastolic dysfunction in HF and under conditions of increased ROS production.\textsuperscript{16,90} and it may even lead to cellular hypercontracture.\textsuperscript{96}

AP-clamp experiments revealed that late $I_{Na}$-dependent Na entry also substantially contributes to arrhythmias in long QT syndrome 3.\textsuperscript{97} Consistent with this evidence, blocking late $I_{Na}$ and Ca entry via NCX have been shown to inhibit afterdepolarizations.\textsuperscript{25,98}

What are the mechanisms that lead to increased late $I_{Na}$? ROS have been shown to increase [Na], prolong AP,
and induce EADs, and ROS-enhanced late $I_{Ca}$ may be involved.16,25,91 Ahern and colleagues showed that nitrosylation of Na-a1.5 increased late $I_{Na}$ under physiological and pathophysiologic conditions.99 Over recent years, however, a body of evidence suggests that CaMKII associates with and phosphorylates Na-a1.5, leading to increased late Na current, and some ROS and nitric oxide effects may be mediated via CaMKII activation (Figure 6).15,16,60,103–104

Because late $I_{Na}$ is strongly linked to cardiac arrhythmogenesis, strategies aimed at inhibiting late $I_{Na}$ may have strong antiarrhythmic potential and may possibly be used to prevent SCD. The clinically approved antianginal drug ranolazine was found to inhibit late $I_{Na}$105,106 within the therapeutic range, which varies between 2 and 8 μmol/L, ranolazine also inhibits $I_{Ca}$ by 30%, which could limit Ca entry,106 and $I_{Kr}$, which can prolong APD. Depending on the actual ionic conditions, the effects of ranolazine on APD may vary. Inhibition of late $I_{Na}$ by ranolazine has been shown, for instance, to reduce ROS-dependent AP prolongation, as well as intracellular Na and Ca accumulation, diastolic dysfunction, and arrhythmias.16,25,90,107,108 Ranolazine has been demonstrated to inhibit afterdepolarizations, to reduce transmural dispersion of repolarization, and to inhibit spatially discordant repolarization alternans in various models of enhanced late $I_{Na}$95,105,106,109–111. It also prevented pacing-induced reentry and multifocal VF.112 In experimental models of systolic HF, ranolazine treatment also improved systolic function,113 possibly by reducing late $I_{Na}$-dependent diastolic SR Ca leak.19

The clinical safety and efficacy of ranolazine are well investigated. A large clinical outcome trial investigating the efficacy of ranolazine to reduce cardiovascular death, myocardial infarction, or recurrent ischemia in >60000 patients with acute coronary syndrome (Metabolic Efficiency With Ranolazine for Less Ischemia in Non-ST-Elevation Acute Coronary Syndrome Thrombosis in Myocardial Infarction 36 trial [MERLIN-TIMI-36]) was not able to show an improvement in outcome.114 Nevertheless, subgroup analysis revealed that in placebo-treated patients, prolonged QTc was a significant independent predictor for SCD, whereas this was not the case in patients treated with ranolazine,115 suggesting a potential beneficial effect in the prevention of SCD. Investigation of the 7-day Holter monitoring data acquired in the MERLIN-TIMI-36 trial revealed a reduction in the incidence of ventricular tachycardia in the ranolazine treated patients.116 Ranolazine also reduced VT burden and implantable cardioverter-defibrillator shocks in a small observational study of patients with refractory VT and previous implantable cardioverter-defibrillator shocks.117 In a small study of patients with long QT syndrome 3, ranolazine shortened the QTc interval in a concentration-dependent manner.118 Moreover, in patients with atrial fibrillation, a dose-finding randomized controlled trial showed a decreased rate of in overall atrial fibrillation recurrence.119

CaMKII

CaMKII is a central regulator of many Na and Ca (and K) channels and transporters. It is well established that transgenic overexpression of CaMKII results in the development of HF and arrhythmias115,120 and that CaMKII inhibition prevents afterdepolarizations and arrhythmias on β-adrenergic stimulation.18

Increased CaMKII autophosphorylation was observed in rabbit chronic atrioventricular block models of left ventricular dysfunction, acquired long QT syndrome, and incessant ventricular tachycardia.121,122 In addition, CaMKII inhibition prevents the development of structural heart disease or arrhythmias on myocardial infarction,123 increased ROS formation,124 pressure overload,125 or pacing-induced incessant VT.122 Calcinurin overexpressing mice with increased CaMKII activity show contractile dysfunction and arrhythmias.126 Also here, CaMKII inhibition improved contractile function and suppressed arrhythmias.127

Oxidized CaMKII has been shown to be involved in ROS-induced enhancement of late $I_{Na}$ (Figure 6), leading to intracellular Na and Ca accumulation, contracture dysfunction, and triggered activity.16,126 Increased oxidative activation of CaMKII because of enhanced NADPH oxidase 2 may also be important for angiotensin II-dependent arrhythmogenesis.127,128 Moreover, oxidized CaMKII plays a role in diabetes mellitus-dependent increased mortality after myocardial infarction possibly by sinus node dysfunction.129 A novel CaMKII activation pathway by O-linked glycosylation at serine 279 was demonstrated during acute hyperglycemia. This activation confers similar autonomous activity as autophosphorylation or oxidation.130 O-linked glycosylation on diabetic hyperglycemia was shown to result in increased diastolic SR Ca leak and be proarrhythmogenic.130 CaMKII can also be nitrosylated at another site in the regulatory domain to cause autonomous activation.131 Nitrosylation-dependent CaMKII activation has been implicated in increased SR Ca leak in cardiac myocytes.132,133 Neurohumoral activation that occurs in HF has been shown to activate CaMKII. Both β-adrenergic stimulation and angiotensin II exposure can activate CaMKII either by increased cytosolic Ca or by increased ROS.12,123,127,134–137

The mechanisms of CaMKII-dependent arrhythmias are complex because CaMKII regulates a variety of ion channels and transporters (Figure 6). CaMKII was first identified to increase peak L-type Ca current and slow $I_{Ca}$ inactivation,138–140 which may predispose to EADs.141–143

CaMKII is also known to associate with and phosphorylate cardiac Na-a1.5,15,16,60,100–104. Beside increased late $I_{Na}$ that may contribute to AP prolongation, increased EAD and DAD formation, and repolarization alternans, CaMKII-dependent phosphorylation also enhanced $I_{Na}$ intermediate inactivation and slowed recovery from inactivation,15,60,103,104. Both effects reduce the steady-state availability of $I_{Na}$, which could lead to repolarization alternans at high heart rates. Reduced Na channel availability can also increase transmural dispersion of repolarization and slow intraventricular conduction (Figure 7).15 This peculiar phenotype of CaMKII-dependent gain in Na channel function (enhanced late $I_{Na}$) and loss of Na channel function (reduced $I_{Na}$ availability) may sound incongruous. However, similar changes in $I_{Na}$ gating have been observed in a SCN5A mutation (1795InsD) associated with features of both Brugada and long QT syndrome.144,145 Thus, increased CaMKII activity in HF may lead to an acquired form of combined long QT and Brugada syndrome.

There is an overwhelming body of evidence that CaMKII regulates RyR2 and SERCA2a. Although RyR2 is directly
phosphorylated at serine 2814,146 the activity of SERCA2a is indirectly influenced by phosphorylation of phospholamban (PLN) at threonine 17. The latter results in relief of PLN-dependent SERCA2a inhibition and activation of SERCA2a.147 In HF, increased diastolic Ca leak through RyR2 occurs in the face of a reduced SR Ca reuptake because of a smaller SERCA2a/PLN expression ratio. This results in a reduced SR Ca content, which is an important determinant for the impaired Ca transient amplitude of failing cardiomyocytes.7,148,149 In HF, CaMKII-dependent diastolic SR Ca leak is a major cause for DADs by activating forward mode NCX.150,151 Zhang et al152 tried to rescue cardiac function in CaMKII δ transgenic with reduced SR Ca load by PLN ablation. Although this improved SR Ca load and Ca transients, this occurred at the expense of increased CaMKII-dependent SR Ca leak, mitochondrial Ca loading, and myocyte death. Thus, despite improved myocyte function, cardiac function, HF progression, and mortality were worsened by the PLN ablation.

More insights into the role of CaMKII-dependent SR Ca leak for contractile dysfunction and arrhythmias in HF comes from animal models with increased afterload or myocardial infarction. It was shown that afterload-induced HF development and arrhythmias were inhibited in knock-in mice harboring only a mutant RyR2 (S2814A) that cannot be phosphorylated by CaMKII at that site.153,154 This knock-in, however, did not prevent HF postmyocardial infarction.153 In contrast, recent data show similar CaMKII-dependent SR Ca leak in isolated human ventricular cardiomyocytes from ischemic and dilated cardiomyopathy.155 Also, KI mice with a CaMKII-phosphomimetic mutation (S2814D) exhibited increased SR Ca leak and developed sustained ventricular tachycardia and SCD on β-adrenergic stimulation, programmed electric stimulation, and increased afterload.154 This sort of CaMKII-dependent SR Ca leak has also been observed on β-adrenergic stimulation156–158 and by activation of NADPH oxidase resulting in DADs.127 Although much attention has been on ventricular muscle, it was also shown that CaMKII-dependent SR Ca leak is crucially involved in atrial arrhythmogenesis.159–162 For ROS-induced SR Ca leak, however, a direct oxidation of the RyR2 has also been proposed.16,163–165 Thus, CaMKII inhibition may be a novel strategy to prevent SCD.

**Catecholaminergic Polymorphic Ventricular Tachycardia**

Increased SR Ca leak is causally involved in catecholaminergic polymorphic ventricular tachycardia (CPVT), a rare familial condition characterized by ventricular arrhythmias that are associated with exercise or catecholaminergic stimulation in a structurally normal heart.166 CPVT occurs at young ages. For example, the majority of 101 patients with CPVT had symptoms before the age of 21.167 Importantly, CPVT

**Figure 7. Ca/calmodulin-dependent kinase II (CaMKII)–dependent regulation of \( I_{Na} \) gating and alternans.**

A, CaMKII-dependent phosphorylation of \( \alpha_{Na} \) has been shown to enhance \( I_{Na} \) intermediate inactivation (reproduced from Wagner et al14 with permission; whole-cell patch clamp in rabbit ventricular myocytes). As a result, the number of available Na channels is reduced especially at shorter diastolic intervals. B, Consequences of enhanced \( I_{Na} \) intermediate inactivation: CV and repolarization alternans. Reduced Na channel availability results in slowed intramural conduction and slowed AP upstroke velocity (1) evident as broader QRS complex on surface ECG (1). In addition, K channel expression is larger in epicardium. Therefore, repolarization is faster in epicardium, especially if Na current is reduced (2). This leads to increased transmural dispersion of repolarization evident as larger and wider T wave on surface ECG (2). Interestingly, Na channels in intermediate inactive state cannot be activated (and become refractory) during the excitation. Thus, these channels are available for the consecutive excitation. Consequently, conduction velocity and AP upstroke velocity will be larger, AP duration longer for the consecutive excitation: the typical pattern of CV and repolarization alternans.
accounts for 15% of all sudden unexplained deaths in young people. CPVT is linked to mutations in RyR2 and the intra-SR Ca binding and RyR2-associated proteins calsequestrin (CASQ2) and triadin (TRND).168–172 For a substantial fraction of patients, however, the disease-causing gene has remained elusive to date. As a result of the irregular SR Ca release,173 electrical NCX is activated causing DADs. Clinically, patients exhibit monomorphic ventricular premature beats and more severe bidirectional ventricular tachycardia, which can degenerate into polymorphic VT and VF. Interestingly, pharmacological CaMKII inhibition has recently been shown to inhibit stress-induced arrhythmias and triggered activity in a mouse model of CPVT (RyR2 R4496C+/−).174 The opposite, transgenic CaMKII overexpression in RyR2 R4496C−/− mice resulted in increase SR Ca leak, DADs, arrhythmias, and increased mortality possibly because of SCDC.175 Because disturbed CaMKII-dependent regulation of SR Ca release is a frequent feature of HF, exercise-induced arrhythmias in HF may be related to the same underlying mechanism. For more information about this important genetic arrhythmogenic disease, the reader is referred to these more comprehensive reviews.176–178

**Epigenetic Modifications Related to SCD**

Epigenetic regulation of gene expression is increasingly recognized as important contributor arrhythmias.179 This emerging area includes microRNAs, DNA methylation, and histone modifications (eg, acetylation/deacetylation). Apropos to the Ca/CaMKII focus here, it is known that CaMKII can importantly influence the nuclear export of class II histone deacetylases (eg, HDAC4 and HDAC5) and that it can modulate transcription of key proteins involved in hypertrophic signaling and arrhythmias.180–182 CaMKII can also directly phosphorylate histone H3 and contribute to hypertrophic changes in gene expression.183,184 Thus, CaMKII seems to be integrally involved in epigenetic mechanisms of cardiac hypertrophy, and some of the consequent changes in protein expression (of ion channels and Ca regulatory proteins) may also contribute to enhanced propensity for arrhythmias.

**Conclusions**

Cardiac myocyte Na and Ca fluxes and concentrations are tightly controlled, but also change dynamically as part of normal physiological modulation of cardiac electric, contractile, and energetic state. There is tight coupling between Na, Ca, electric, mechanical, and energetic properties in the heart, only some of which we touched upon here. Many arrhythmias are a result of dysregulation of this complex system. To understand how deranged Na and Ca homeostasis comes about in pathophysiological states and how it contributes to electric and contractile dysfunction, such as HF, arrhythmias and SCD, is a challenge. But this understanding is essential for progress in novel therapeutic approaches to these major clinical problems.

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**References**


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