# PATHOPHYSIOLOGY OF CARDIAC ARRHYTHMIA

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# Pathophysiology of Cardiac Arrhythmia

**ANCC Accredited NCPD Hours: 1.4 hrs**

**Target Audience: RN/APRN**

## Need Assessment

Arrhythmias are common but can be life threatening and early diagnosis is essential to reduce mortality. The most common arrhythmia is atrial fibrillation and may cause stroke if left inadequately treated. It is estimated that between 2.7 million and 6.1 million people in the United States have atrial fibrillation. Nurses' knowledge and practices regarding life-threatening arrhythmias need to be improved and enrichment of nurses' knowledge and practice regarding lethal arrhythmia detection and management is highly recommended.

## Objectives

- Discuss the involvement of Na+/Ca2+ exchanger (NCX) in the generation of cardiac arrhythmia
- Describe the pathophysiological concept of cardiac arrhythmia
- Identify two reasons for the antiarrhythmic potential of acute NCX inhibition
- Discuss the pathogenesis of cardiac arrhythmia
- Describe arrhythmias due to Long QT syndrome (LQTS)

## Goal

The goal of this article is to investigate the adaptive mechanisms and complex pathophysiological aspects of cardiac arrhythmia

## Introduction

The great majority of cardiac disorders are associated with at least one of three main pathophysiology: arrhythmia, ischemia and heart failure. In clinical settings these entities often occur in combination sometimes mutually reinforcing and/or depending upon one another. As a clinical example, ***human heart failure renders the heart more susceptible to arrhythmia.*** Conversely, ***ischemia can result in heart failure and increased arrhythmia burden. Both extracellular and cellular mechanisms have been identified in the causal chains leading to heart failure, arrhythmia or ischemia*** and some of these have been successfully identified as therapeutic targets [1, Rank 5]

> "Any rhythm in the heart that falls outside the norm with respect to rate, regularity, and propagation sequence of depolarization wave is known as arrhythmia. In an arrhythmia the heartbeats may be too slow, too rapid, too irregular, or too early."

## Etiology of Arrhythmia

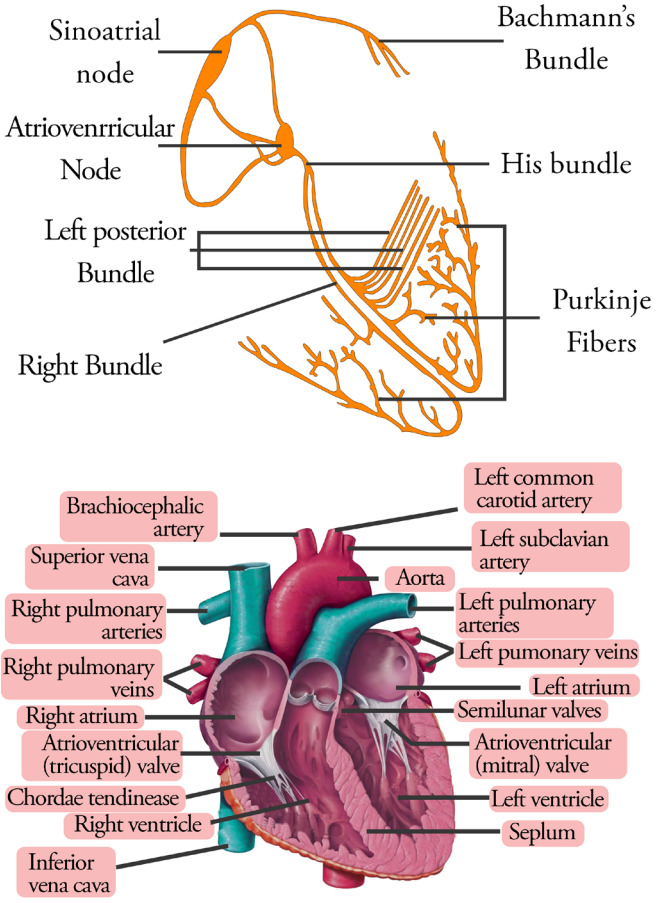


Figure 1: Heart

There are three common arrhythmia etiologies:

***Automaticity*** results from alterations of the basic cellular ion exchange mechanism which is depicted as a distinct electrical pattern, the action potential. Once an electrical impulse is initiated, it typically propagates cell to cell in a relatively uninterrupted fashion. ***Automaticity is the ability to depolarise spontaneously.***

There are structures in the heart that have the ability for automaticity.

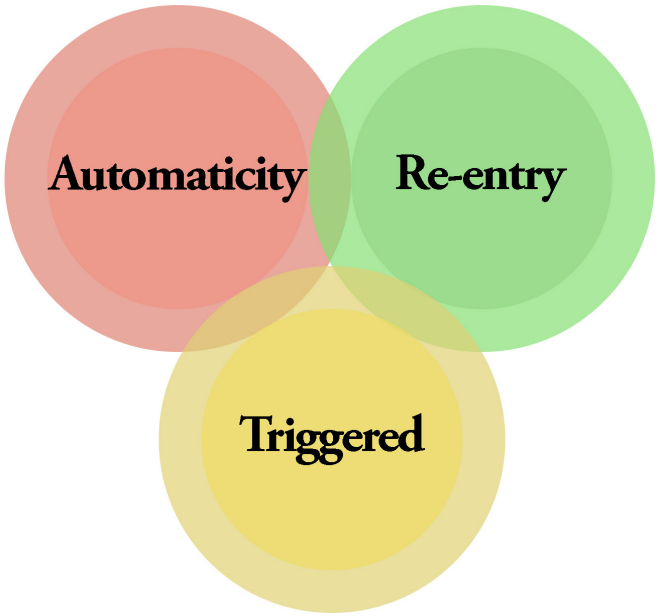


Figure 2: Etiology of Arrhythmia

If an obstruction of valves, veins or post-infarction scar tissue occurs, the impulse can circle around the obstruction, creating a re-entrant pathway. Drugs or disease can alter cell action potentials, triggering abnormal impulse initiation.

***Triggered*** Action occurs after depolarisation, it is an action potential which is engendered by an after depolarisation which causes extrasystoles.

***Re-entry*** occurs if the depolarising impulse encounters a blocked area that can only be passed on one side. It means that the depolarising wave front moves around as an electrical circle loop through a path of electrically connected myocardium. This circuit must surround a core of tissue that cannot be depolarised.

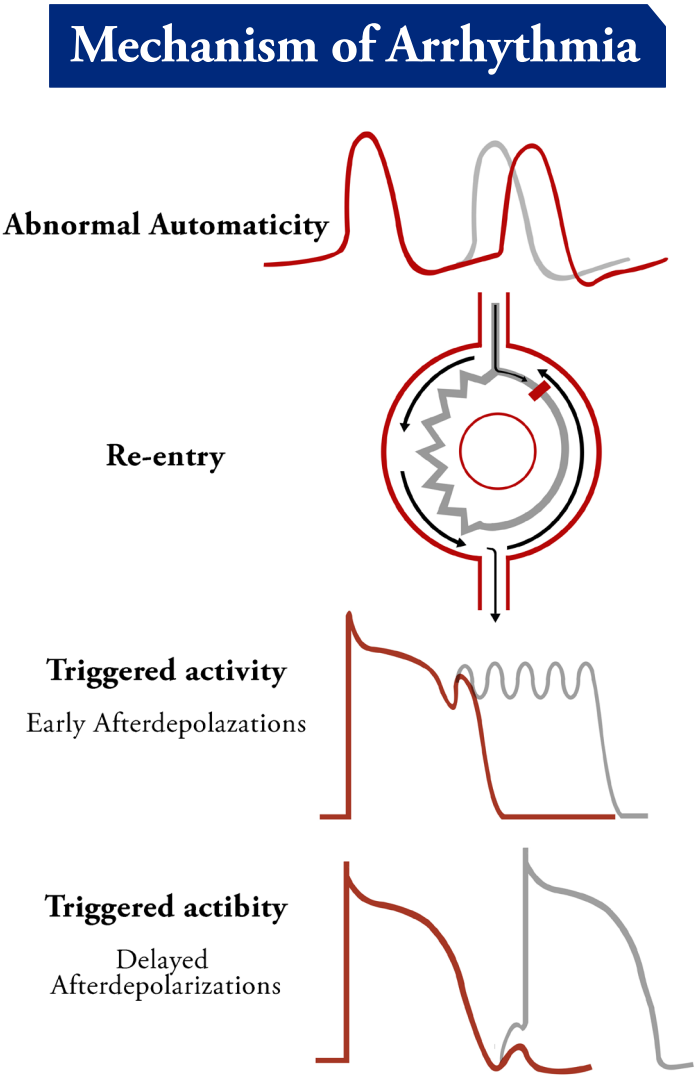


Figure 3: Arrhythmia Etiological Processes

> "The SA node, atrial myocardial cells located around crista terminalis, coronary sinus entrance, inferior venacava, cells around mitral and tricuspid valves, cell clusters around AV node, bundle of His and entire Purkinje fibres possess Automaticity"

Mechanisms responsible for cardiac arrhythmias are generally divided into 2 major categories:

1. Enhanced or abnormal impulse formation - Focal activity
2. Conduction disturbances - Re-entry

## Abnormal Impulse Formation

It can be due to automaticity or triggered activity. The Sino Atrial (SA) node displays the highest intrinsic rate of approximately70 depolarisations per minute and all other pacemakers are known as subsidiary or latent pacemakers because they initiate excitation of the heart only when the SA node is unable to generate impulses or when fail to propagate.

Calcium clock is activated by hyperpolarization rather than depolarization At the end of the action potential, the pacemaker current is activated and depolarizes the sarcolemmal membrane. Another important ionic current capable of depolarizing the cell is the sodium-calcium exchanger current. Studies suggest that sympathetic stimulation accelerates heart rate by phosphorylation of proteins that regulate calcium ion balance and spontaneous Sarcoplasmic reticulum- SR Ca cycling.

> **"SA node automaticity can be described by Sarcolemma voltage or Calcium clocks which refer to voltage-sensitive membrane currents, such as the hyperpolarization-activated pacemaker current."**

## Subsidiary Pacemakers

In addition to the SA node, the atrioventicular (AV) node and Purkinje system are also capable of generating automatic activity. If the impulse generation or conduction in the SA node is impaired, latent or subsidiary pacemakers within the atria or ventricles take control of pacing the heart. The intrinsically slower rates of these latent pacemakers generally result in bradycardia. Both atrial and AV junctional subsidiary pacemakers are under autonomic control, with the sympathetic system increasing and parasympathetic system slowing the pacing rate. Although ***acetylcholine produces little in the way of a direct effect, it can significantly reduce Purkinje automaticity by means of the inhibition of the sympathetic influence, a phenomenon termed accentuated antagonism.*** [31]

Bradycardia can occur in structurally normal hearts because of genetic mutations

> **"Ion channels on the surface membrane of sinoatrial nodal pacemaker cells (SANC) are the proximal cause of an action potential. And the group of the ion channel currents reconstructed in silico generates rhythmic action potentials. Thus, this ensemble refers to "membrane clock" (M clock)."**

that result in abnormalities of either membrane clock or Ca clock mechanisms of automaticity.

## After-Depolarization and Triggered Activity

***Two subclasses are traditionally recognized:***

1. ***Early after depolarisation***
2. ***Delayed after depolarisation***

Early afterdepolarization (EAD) interrupts or retards repolarization during phase 2 and/or phase 3 of the cardiac action potential, whereas delayed afterdepolarization (DAD) occurs after full repolarization. When EAD or DAD amplitude suffices to bring the membrane to its threshold potential, a spontaneous action potential referred to as a triggered response is the result. EADs develop more commonly in midmyocardial M cells and Purkinje fibers than in epicardial or endocardial cells when exposed to action potential duration (APD)-prolonging agents. DADs and DAD-induced triggered activity are observed under conditions that augment intracellular calcium, [Ca2+], such as after exposure to toxic levels of cardiac glycosides (digitalis) or catecholamines. This activity is also manifest in hypertrophied and failing hearts as well as in Purkinje fibers surviving myocardial infarction. In contrast to EADs, DADs are always induced at relatively rapid rates. [31]

## The Na+/ Ca2+ (NCX) Exchanger and Cardiac Arrhythmia

The Na+/Ca2+ exchanger (NCX) is the main Ca2+ extrusion mechanism of the cardiac myocyte and thus is crucial for maintaining Ca2+ homeostasis. Increased NCX activity has been identified as a mechanism promoting heart failure, cardiac ischemia and arrhythmia.

***It has been hypothesized that increased NCX activity could induce after depolarizations of the ventricular AP that could then trigger tachyarrhythmias. After depolarizations are defined as oscillations of the electric membrane potential during (early after depolarization***

***[EAD]) or after (delayed after depolarization [DAD]) the cardiac action potential. After depolarizations can induce reentry and are commonly regarded as a major trigger of cardiac tachyarrhythmias.*** [6, Rank 3]

EADs has been shown to occur in animal models with slowed AP repolarization. The prolonged AP duration prolongs the period of time the myocytes spend in a depolarized state which again will broaden the time window for voltage dependent INa or ICa to reactivate and induce an EAD. This mechanism has been evoked to explain proarrhythmia in animal models of hypertrophy and heart failure where a reduction of K+ carried outward currents and an increased AP duration have been observed. Likewise, specific mutations of K+ channel subunits resulting in loss of function, or Na+channel gain-of-function mutations, can both slow AP repolarization and cause EADs (early after depolarization) resulting in ventricular tachyarrhythmia. ***Since NCX also slows repolarization it may be a further candidate for the promotion of EADs.*** [7, rank 4]

To understand the involvement of NCX in the generation of cardiac arrhythmia, it is necessary to define the role that NCX plays in the repolarization of the cardiac AP. During systole, when cytosolic

> **"Depolarizations that attend or follow the cardiac action potential and depend on preceding transmembrane activity for their manifestation are referred to as after-depolarizations."**

Ca2+ concentration is high, NCX extrudes Ca2+ from the cytosol into the extracellular space. Due to the electrogenic nature of this process, NCX generates an inwardly directed membrane current (INCX) during SR Ca2+ release. It has been assumed that via this mechanism NCX would contribute to maintain the plateau of the cardiac action potential.

***NCX is also essential for the development of DADs*** (Delayed After Depolarization). The initiating event of a DAD consists of a spontaneous release of Ca2+ from the SR, as occurs in the setting of SR Ca2+ overload. This sudden increase of cytosolic Ca2+ then leads to an activation of NCX inward current which depolarizes the sarcolemmal membrane. If this reaches the threshold for Na+ channel activation, a new and premature AP is triggered. [8, Rank 5]

Initial studies in isolated cardiac myocytes from several different species have indeed demonstrated that delayed after depolarization can be triggered via Ca2+

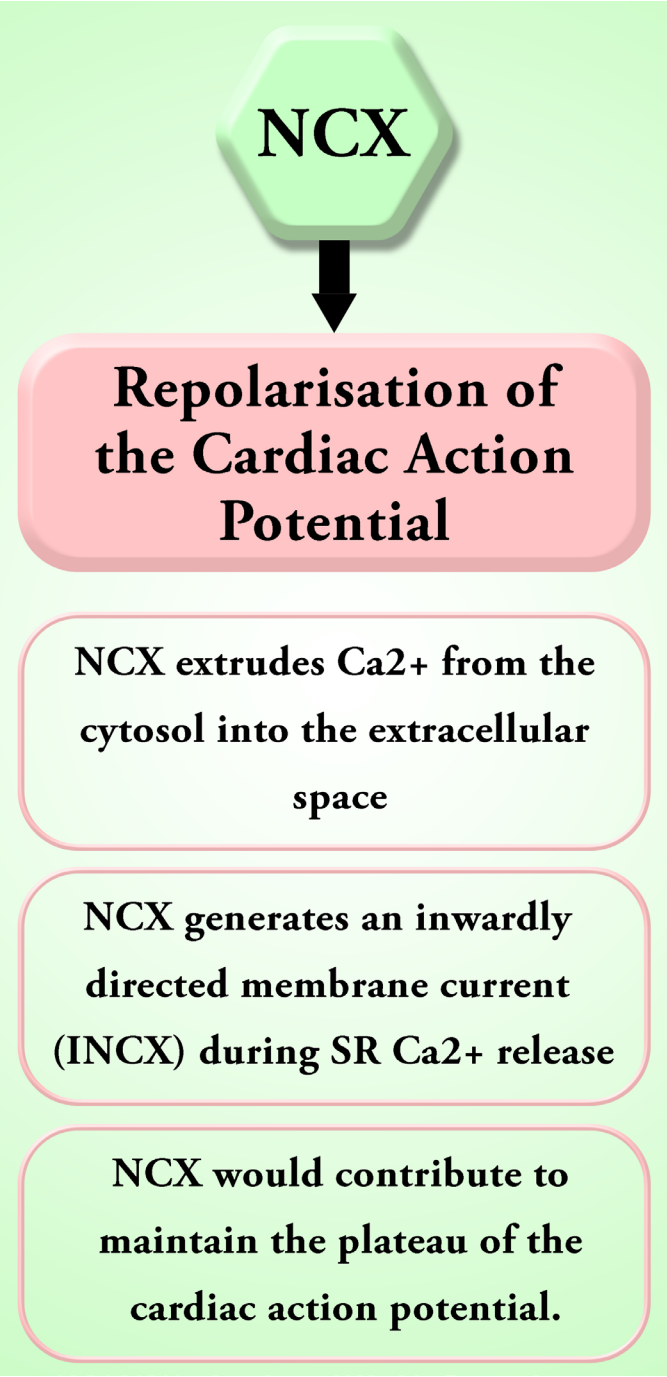


Figure 4: Function of NCX in Cardiac Cycle

overload of the SR. An increased occurrence of after depolarizations of the cardiac AP has also been observed in animal models of hypertrophy and heart failure, where NCX is known to be overexpressed. [9, rank 3]

***Studies testing the proarrhythmic potential of increased NCX expression have mostly been limited to hypertrophied and failing myocardium.*** Therefore, their informations on the role of NCX in the generation of cardiac arrhythmia are somewhat limited because a multitude of structural, expressional and functional changes other than the upregulation of NCX have been observed in human and animal failing and hypertrophic myocardium and any one or a combination of these could be potential promoters of cardiac arrhythmia. The NCX overexpressing mouse on the other hand has increased NCX activity in the absence of cellular hypertrophy or reduced K+ channel activity. Before 14 weeks, these animals have normal whole heart and cellular function and structure and preserved K+ outward currents. These animals are prone to whole heart ventricular tachyarrhythmia. ***In isolated cardiac myocytes, APs are prolonged and there is an increased occurrence of EADs as well as DADs.*** These observations indicate that NCX may be a promoter for arrhythmia independent of structural or ionic remodelling associated with heart failure. [10, Rank 2]

## Na+ Channel

Expression of ion channels in heterologous systems allows for investigation of inherited ion channel defects at the single-protein and cellular levels to directly identify the disease-associated alteration in ion channel function. Disease-linked mutations provide an opportunity to understand the mechanistic basis of human disease from altered molecular function to the clinical syndrome. Initial investigation of SCN5A (Sodium Voltage-Gated Channel Alpha Subunit 5) mutations linked to LQT-3 (Long QT -3) syndrome revealed striking mutation-induced defects in channel behavior that were consistent with the disease phenotype. Classically, Na+ channel activation is associated with the spread of depolarization in the heart that underlies the QRS complex of the ECG. In the case of LQT-3 syndrome, however, defects in the Na+ channel were linked to delay in ventricular repolarization or prolongation of the QT interval. These initial experiments clearly showed a novel mechanism that could explain this unexpected result: the inherited mutation disrupted the inactivation process of the channel such that, during the plateau phase of the action potential, a small number of Na+ channels do not inactivate or become non-conducting but in fact reopen to provide a very small depolarizing current. Even though this late current is only a fraction of the total Na+ channel current responsible for the QRS complex, this small disease-associated perturbation in plateau current is responsible for prolonging the QT interval in mutation carriers and raising the risk of cardiac events. Thus, the molecular genetic analysis of LQTS patients has led to a novel understanding of the importance of Na+ channel activity in controlling not only the QRS complex, but also the duration of the ventricular electrical response: the QT interval. Recently, mutations in SCN1A, (Sodium Voltage-Gated Channel Alpha Subunit 1) the gene coding for the human neuronal Na+ channel α subunit associated with epilepsy, have been reported to cause similar defects in channel-inactivation gating. As yet, the cellular consequences of such epilepsy-associated mutations remain elusive, but mechanistic insights gained from investigation of cardiac defects are very likely to have widespread implications. [13, rank 5]

## K+ Channels

Investigation of the molecular basis of Long QT syndrome has led to fundamental insight into the molecular identity of key K+ channel subunits in the heart,

notably the two key delayed rectifier currents IKr (Rapidly Acting Delayed Rectifier Potassium Current) and IKs (Slowly Acting Delayed Rectifier Potassium Current) which have been demonstrated in animal models to be crucial to control of cardiac action potential duration. In particular, mutations in KCNQ1 (Potassium Voltage-Gated Channel Subfamily Q Member 1), which codes for the α subunit of the IKs channel, cause LQT-1 syndrome, and mutations in KCNE1 (Potassium Voltage-Gated Channel Subfamily E Regulatory Subunit 1), the gene coding for the auxiliary β subunit of the IKs channel, cause LQT-5 syndrome. It had been well established in animal models that IKs is strongly regulated by SNS (Sympathetic Nervous System) stimulation, such that SNS stimulation increases reserves of repolarizing current and contributes to the shortening of action potential that occurs in parallel with the SNS stimulation–induced increases in heart rate.

Further, clinical data indicate that carriers of mutations in either KCNQ1 or KCNE1 are at increased risk of experiencing a fatal cardiac arrhythmia in the face of elevated SNS activity. Together, these findings motivated investigation into molecular links between the SNS and regulation of KCNQ1/KCNE1 channels in the human heart. This work resulted in the discovery that the KCNQ1/KCNE1 channel actually forms a macromolecular signalling complex: coupled to the carboxy-terminal domain of the channel is an adaptor protein, yotiao, which in turn binds to the regulatory enzymes protein kinase A (PKA) and protein phosphatase 1 (PP1). Thus the channel, via the adapter protein, recruits enzymes that can up-regulate (PKA) and down-regulate (PP1) channel activity by phosphorylation and dephosphorylation of a serine in its amino-terminal domain. When this complex is disrupted, the channel is not properly regulated and there is imbalance in control of the ventricular action potential, which leads to high risk of arrhythmia. Because other targets of protein kinase A (PKA), such as the ryanodine receptor of the sarcoplasmic reticulum, form independent macromolecular signalling complexes, selective disruption of the KCNQ1/ KCNE1 signalling complex by inherited mutations may disrupt a micro-molecular signalling domain restricted to one channel — a potentially novel mechanism that may mechanistically contribute to the genesis of cardiac arrhythmias. [14, Rank 2]

## NCX Activity and Action Potential (AP) Kinetics

Indeed, ***computer modelling revealed that increased INCX activity significantly delays repolarization of the cardiac AP. Research also demonstrated in cardiomyocytes that an inhibition of NCX caused an abbreviation of the AP while a prolongation was observed when NCX activity was maximal.*** Many groups have used transgenic models with cardiac specific alteration of NCX expression to further define the role of NCX in AP repolarization. A prolonged AP plateau was observed in NCX over expressor mice, and – conversely – a shortened AP in NCX knockout mice, although in knockout mice, additional mechanisms contribute to shortening AP duration. [4, Rank 4]

In addition to a direct influence of NCX inward current on AP repolarization, an alternative mechanism may explain NCX-mediated repolarization changes: a functional interplay between NCX and L-type Ca2+ channels. One of the strongest regulators of ICa is cytosolic Ca2+ itself. Thus, at high intracellular Ca2+ concentrations, Ca2+ binds to calmodulin, thereby inactivating ICa. At low cytosolic Ca2+ concentrations, when no Ca2+ is bound, L-type Ca2+ current is considered to be maximal. ***The DHPR is primarily located at sarcolemmal SR junctions in close vicinity to the RyR and NCX.*** It has been accepted that Ca2+ release from the SR is the primary mechanism to influence and terminate L-type Ca2+ current. However, due to its close vicinity, NCX activity may also influence ICa. Blockade of NCX may increase cleft Ca2+ concentration. This may have important implications for ICa regulation via Ca2+ induced inactivation: ***In the absence of NCX activity elevated cleft Ca2+ decreases*** ICa open probability and accelerates ICa inactivation. Conversely, when NCX activity is high, this may increase ICa open probability and delay ICa inactivation. Indeed, in NCX knockout, ICa is reduced via a Ca2+ dependent mechanism, while – conversely - ICa in NCX overexpressor is increased, a mechanism that also appears Ca2+ dependent. Via this functional interplay with ICa, NCX may – indirectly - influence AP repolarization in the same direction as it does directly via reduced or increased NCX inward current. Thus, increased INCX would also increase net ICa thereby further slowing repolarization. Conversely, reduced INCX would also reduce ICa thus reducing AP duration. [5, Rank 2]

## Experimental Inhibition of NCX to Counter Pro-arrhythmia

Early studies investigating the antiarrhythmic potential of the NCX inhibitor SEA0400- novel and selective inhibitor of the Na+-Ca2+ exchanger yielded inconsistent results. While studies observed that the NCX inhibitor SEA0400 (a novel and selective inhibitor of the Na+-Ca2+ exchanger) inhibited EADs induced by dofelitide and barium in guinea pig cardiac muscle strips, it did not observe a protective effect of SEA0400 in either in vivo studies or isolated cardiac myocytes when monitoring aconitine-induced arrhythmia in the same species. Two independent studies investigated the effects of SEA0400 on arrhythmias induced by ouabain. In an in vivo dog model, studies observed that pharmacological inhibition of NCX protected against ouabain-induced arrhythmia but not against arrhythmia induced by acute ischemia. A recent study has specifically investigated the effect of SEA0400 on cardiac myocytes isolated from the pulmonary vein regions of guinea pig left atria. Spontaneous electrical activity generated by this cell population is regarded to be the trigger for atrial fibrillation, one of the most frequent tachyarrhythmias.

To produce proarrhythmic experimental conditions in which after depolarizations occur, studies utilized a recently developed intact heart model of the rabbit heart mimicking the electrophysiological alterations of long QT 2 and 3 syndromes (LQT 2 and 3). Under these experimental conditions, significantly reduced action potential duration and suppressed EADs and the Torsade-de-Pointes form of ventricular tachycardia. [11, Rank 4]

The discrepancies evident in studies investigating the antiarrhythmic potential of acute NCX inhibition may be due to the following factors:

1. Proarrhythmic interventions of a different nature have been applied (i.e. aconitine-, vs. ouabain-, vs. dofetilide- vs. ischemia-induced arrhythmia)
2. Different species have been investigated and species differences in the composition of repolarizing ion channels are well known.
3. Finally, different preparations of cardiac tissue ranging from in vivo models over trabecular preparations to patch clamp experiments in isolated cardiac myocytes have been used. [12, Rank 3]

## Abnormal Impulse Formation

Reentry is fundamentally different from automaticity or triggered activity in the mechanism by which it initiates and sustains cardiac arrhythmias. Circus movement reentry occurs when an activation wave front propagates around an anatomic or functional obstacle or core, and re-excites the site of origin. In this type of reentry, all cells take turns in recovering from excitation so that they are ready to be excited again when the next wave-front arrives. In contrast, reflection and phase 2 reentry occur in a setting in which large differences of recovery from refractoriness exist between one site and another. The site with delayed recovery serves as a virtual electrode that excites its already recovered neighbor, resulting in a reentrant reexcitation. In addition, reentry can also be classified as anatomic and functional, although there is a gray zone in which both functional and anatomic factors are important in determining the characteristics of reentrant excitation.

## Free combinations of LAMA plus LABA

***A genetic disorder disrupting electrical activity in the heart, congenital Long QT Syndrome (LQTS) can lead to life-threatening arrhythmias and sudden cardiac death.*** The more common, autosomal dominant form of congenital LQTS that presents without deafness was first described in two separate cases and became known as Romano-Ward syndrome. Since these initial patient descriptions, advances in our understanding of the mechanisms of cardiac electrical excitability at the tissue, cellular, and molecular level have yielded much insight into the pathophysiology of congenital LQTS. [15, Rank 4]

Clinically, ***congenital LQTS patients often first present after episodes of syncope and/or seizure, and the ECG reveals a prolonged QT interval.*** The ECG measures electrical activity of the heart over time, at the patient's body surface. The primary electrical signals observed include the P wave, which signifies atrial depolarization; the QRS complex, which arises from ventricular depolarization; and the T wave, due to ventricular repolarization. The QT interval, therefore, reflects the time elapsed from the initiation of ventricular depolarization to the end of ventricular repolarization. The QT interval shortens with increasing heart rate thus requiring a normalization, or correction, for heart rate. For a diagnosis of LQTS, this rate-corrected QT (QTc) interval prolongation on a 12-lead ECG generally is referenced as >470 ms for males and >480 ms

for females. QTc also varies with age, and thus, an age-appropriate prolonged QTc interval in a patient aids the diagnosis of LQTS. However, diagnosis of LQTS based on absolute QT interval cutoffs can be challenging, since there is considerable overlap in the QTc distribution of affected patients and otherwise healthy individuals. Asymptomatic patients can have intervals beyond this cut-off and develop no arrhythmias; similarly, QTc intervals below this cutoff can be seen in patients with established LQTS (with clinical arrhythmias and positive genetic testing). Clinical scoring systems, as well as genetic testing, can be helpful to assist with the diagnosis of congenital LQTS, particularly when QT intervals are on the borderline (within 20 ms of these cutoffs) or when clinical history is equivocal. This review will focus primarily on the various forms of congenital LQTS. [16, Rank 3]

Ion channels are the molecular entities underlying most ionic currents in the heart, allowing passive diffusion of ions across the cell membrane's electrochemical gradients. A selectivity filter in the channel pore, determined by distinct atomic components, endows selective permeation of ions, such as Na+, K+, and Ca2+. Some ion channels exhibit voltage-dependent gating, where voltage-sensing domains respond to changes in membrane potential to cause channel opening or closing. [17, Rank 1]

## Mutation-induced Ion Channel Dysfunction

***The ventricular action potential of the human heart is distinct in that the temporal period separating excitation of ventricular cells from relaxation, or repolarization, is very long, typically on the order of 450 milliseconds.*** This timing is crucial because as long as the ventricular tissue is depolarized it cannot be re-excited due to the unavailability of key voltage-gated Na+ channels which normally enter a nonconducting inactivated state during this period. The duration of this depolarized state, often referred to as the plateau phase of the ventricular action potential, is not only cardioprotective against premature excitation but is also essential to maintaining the proper timing between diastolic filling and ejection intervals. It is the plateau phase of the ventricular action potential that thus determines the QT interval of the ECG. This crucial plateau phase is maintained by a delicate balance of small ionic conductances, which, though energetically favorable, predispose this period of the electrical cycle in the heart to disturbances that may be caused by otherwise harmlessly small changes in

transmembrane ionic activity. [2, Rank 3]

Before genetic information was successfully used to identify genes associated with congenital LQTS, various candidate ion channels (and thus channel-coding genes) were suspected to underlie the clinical defect of delayed ventricular relaxation. ***Molecular genetic approaches removed at least some of the uncertainty by demonstrating a clear association between mutations in genes encoding specific ion channel subunits and LQTS.*** Research has revealed that ***mutations in at least five genes coding for key cardiac K+ channel subunits result in a loss or reduction of channel activity that can cause variants of LQTS.*** Surprisingly, mutations in the principal (α) subunit of a key heart Na+ channel (encoded by SCN5A) that, in general, result in a gain of channel function also cause yet another variant of LQTS, known as LQT-3 syndrome. These discoveries have prompted at least three new areas of research that impact not only the diagnosis and management of LQTS, but also our understanding of human cardiovascular physiology. These include (a) mutation-specific therapeutic strategies (b) identification of mutation-specific risk of cardiac events, and (c) mechanistic insight into the role of altered channel function and regulation in the control of QT intervals in the heart. ***Research focus on two areas in which fundamentally novel insight into human physiology emerged from investigation of the mechanistic basis of LQTS:*** the role of altered Na+ channel gating in control of the QT interval, and the molecular link between the sympathetic nervous system (SNS) and electrical signaling in the ventricle. [3, Rank 4]

## Modes of Normal and Pathological Cardiac Regulation by Subcellular Signalling

Obviously, ion channels do not function in isolation. Rather, ion channels and pumps are modulated by elaborate signaling networks. Cell signaling networks regulate multiple aspects of cardiac tissue function, including AP (Action Potential) morphology, calcium handling, contraction, metabolism, and gene regulation. A quantitative understanding of regulation by signaling networks is crucial for appreciating how many cell components interact to produce arrhythmias in both healthy and diseased states but is still a challenge owing to overlapping layers of interdependencies between networks, as well as multiple feedback loops within networks.

A crucial and well-studied player in cell signaling is Ca2+/ calmodulin-dependent protein kinase II (CaMKII),

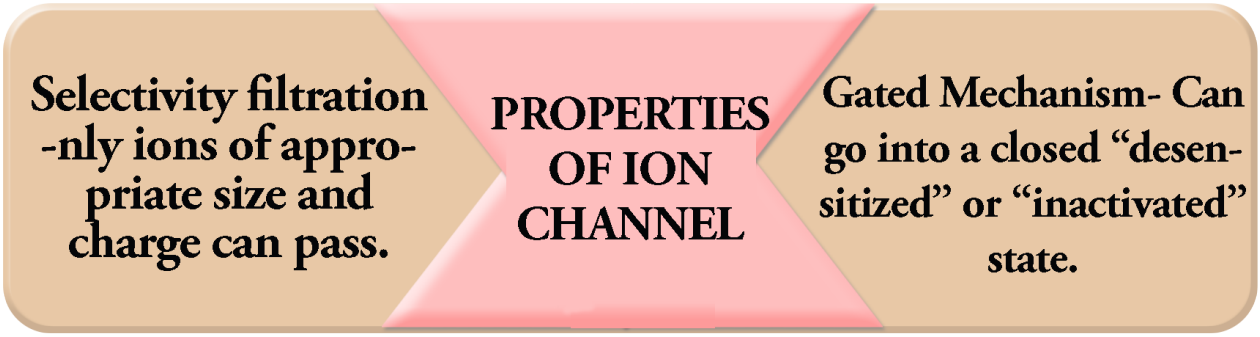


Figure 5: Properties of Ion channels

a multifunctional enzyme that both directly and indirectly modulates the function of several critical cell components, including depolarizing voltage-gated sodium and L-type calcium channels, repolarizing currents Ito, and inwardly rectifying potassium current (IK1), Sarcoplasmic reticulum (SR) calcium handling components ryanodine receptor (RyR) and sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA), and gene transcription within the nucleus. Recent simulation studies using models that include CaMKII (Ca2+ /calmodulin-dependent protein kinase II)signaling have complemented experiments and yielded mechanistic insights into relationships between CaMKII and increased arrhythmia susceptibility caused by altered ion channel dynamics, reduced CV leading to conduction block, and impaired calcium cycling in the ischemic border zone. Researchers have also recently developed models of INa, L-type Ca2+ current (ICa,L), and RyR leak modulation by CaMKII, investigating links to arrhythmogenesis. [18, Rank 4]

The β-adrenergic signaling cascade, which modulates some of the same targets as CaMKII, plays critical roles in the regulation of contractility, metabolism, and gene regulation. β- Receptor activation leads to increased levels of cAMP (Cyclic adenosine monophosphate) within the cell, which then promotes dissociation of PKA(protein kinase A), enabling it to phosphorylate and modulate multiple targets and processes including INa, IKs, RyRs, SERCA, the Na-K pump, troponin I, glycolytic metabolism, and cross-bridge formation. [19, Rank 1]

B-Adrenergic signaling has been implicated in multiple arrhythmia diseases including catecholaminergic polymorphic ventricular tachycardia resulting from abnormal calcium cycling because of increased β-adrenergic tone and LQT1 [caused by modulation of IKs, disrupting repolarization with proarrhythmic consequences]. β-Blockade is commonly prescribed to reduce the likelihood of arrhythmia in patients with LQT, but a recent

modeling study calls into question the validity of this treatment strategy for a subset of patients with LQT and suggests that β-blockers may actually be proarrhythmic in patients with LQT3. [20, Rank 4]

Several groups have used computational modeling to explore the links between β-adrenergic signaling, excitation-contraction (EC) coupling, genetic mutations, and arrhythmogenesis. In one study, it was found that a known gene mutation implicated in LQTS was not sufficient to cause QT prolongation on its own but required β-adrenergic stimulation. When this model was implemented in a three-dimensional (3-D) rabbit ventricular wedge framework, there was increased transmural dispersion of repolarization and T-wave abnormalities on simulated ECGs. [21, rank 2]

More recently, computational models have incorporated signaling by both PKA, which participates in a feedback loop with β-adrenergic receptors via cAMP, and CaMKII. These two pathways engage in significant cross talk, though there are still open questions related to specific interactions. This model represents dual phosphorylation of ICa,L, RyR, and phospholamban by PKA and CaMKII, as well as additional phosphorylation of additional targets by either kinase. Using this model, researchers found that β-adrenergic enhancement of calcium transients was assisted by a synergistic relationship between PKA and CaMKII-dependent phosphorylation of multiple targets. A separate model incorporating both PKA and CaMKII signaling introduced two distinct β-adrenergic receptor isoforms. In this study, the authors used their model to shed insight on control of local Cyclic adenosine monophosphate levels and receptor-specific modulation of AP and calcium transient properties. The model also predicted that isoform-specific reduction of β-adrenergic receptor activation, reduced compartmentation of cAMP, and CaM KII inhibition will reduce the occurrence of afterdepolarizations during β-adrenergic stimulation. [22, Rank 5]

As stated above, the mechanisms by which the PKA and CaMKII pathways communicate with one another are still unresolved. However, computational models are particularly well suited for tackling this type of problem, as links between any two components in the system can be selectively ablated or augmented. One could selectively ablate phosphorylation of phospholamban by either PKA or CaMKII in the Soltis-Saucerman model and assess whether the synergistic inotropic responses are still intact. Or perhaps the amount of

cross talk depends on the amount of available calcium. Calcium availability can be easily reduced in a computational model and can be implemented experimentally with calcium buffers. [23, Rank 3]

## Ischemia Reperfusion Injury and NCX

During cardiac ischemia, a rise in intracellular Na+ occurs due to a reduction of Na+/K+ ATPase activity. This increase in Na+ concentration is followed by a rise in intracellular Ca2+. This accumulation of cytosolic Ca2+ concentration is dependent on the rise in cytosolic Na+ concentration and it has therefore been suggested that the process of Ca2+ accumulation during ischemia is mediated via NCX working in reverse mode. As mentioned above, depending on the transsarcolemmal Na+ and Ca2+ gradients and the membrane potential, NCX can switch into reverse mode and transport Ca2+ into the cell instead of extruding Ca2+. In reverse mode, NCX extrudes 3 Na+ in exchange for 1 Ca2+ ion entering the cell. NCX reverse mode is thus favored by the high cytosolic Na+ concentration characteristic for ischemic conditions. Consistent with this hypothesis, mice with heterozygous overexpression of NCX exhibit increased susceptibility to ischemia-reperfusion injury.

However, during ischemia, there is also a rise in cytosolic H+ concentration that contributes to the rise in cytosolic Na+ concentration via activation of the Na+/H+ exchanger (NHE). This increased Na+ favors NCX reverse mode and cytosolic Ca2+ accumulation. Inhibition of NHE blocks the rise in Na+ and Ca2+and is cardioprotective during ischemia. Some studies have suggested that preventing the rise in cytosolic Na+ concentration via NHE inhibition may be cardioprotective, independent of the effect on Ca2+ accumulation. Both cytosolic accumulation of Ca2+ and Na+ are inhibited by NHE inhibitors, and it is therefore difficult to distinguish between the relative protective effects of preventing the increase in Na+ vs. Ca2+ concentration. [24, Rank 3]

The development of transgenic and genetic transfection techniques has provided a unique tool to further investigate this question. We have demonstrated that NCX KO mice are significantly less susceptible to ischemic injury. Cellular studies have demonstrated that there is no detectible Ca2+entry during experimental lowering of the trans-sarcolemmal Na+ gradient in NCX KO myocytes while WT myocytes exhibited significant cytosolic Ca2+ accumulation under these conditions. During ischemia-reperfusion, hearts from NCX KO mice exhibited less necrosis, better

post-ischemic recovery of cardiac performance, higher levels of high-energy phosphates, and improved recovery of Na+ homeostasis when compared to WT animals. Acute targeted genetic suppression of NCX from isolated cardiac myocytes can also protect from ischemic injury. Similarly, it was shown that introduction of specific splice variants of NCX into neonatal cardiac myocytes via transfection prevented ischemia induced cytosolic Ca2+ accumulation. These observations add further support to the hypothesis that NCX is a direct mediator of ischemic injury. Moreover, these data indicate that inhibition of NCX seems to be protective independent of a direct inhibition of H+ accumulation via NHE. [25, Rank 4]

## Metabolic Links to Electrical Dysfunction

While much of the preceding discussion has focused on abnormal calcium handling and ion channel function, defects in cell metabolism and mitochondrial function have also been implicated in the genesis of arrhythmias, as well as cardiac glycoside toxicity. It is critically important to better understand mitochondrial function in health and disease, since it contains its own subset of metabolic pathways and

> **"Mitochondria are linked to arrhythmogenesis, as the ATP they produce is necessary for the proper function of several cell components that are critical determinants of electrical activity, including SERCA, the Na-K pump, and L-type calcium channels"**

channels that contribute to or underlie pathologies. The mitochondria also exhibit complex behavior such as traversing waves of membrane depolarization. Some proposed mechanisms for mitochondrial involvement in arrhythmogenesis include reactive oxygen species (ROS)-induced ROS release, altered intracellular calcium dynamics (mediated by the mitochondrial calcium uniporter), and collapse of mitochondrial membrane potential (mediated by the inner membrane anion channel, mitochondrial permeability transition pore, and mitochondrial ATP-sensitive potassium channels), leading to loss of ATP production. [26, Rank 4]

Attention has been focused on sarcolemmal ATP-inactivated potassium efflux channels, as they are believed to be responsible for increased APD heterogeneity during metabolic stress, which creates a substrate that is more arrhythmogenic.

Recent computer simulations in this area have made use of models that investigate the positive feedback loops underlying ROS-induced ROS release, as well as detailed models of mitochondrial metabolism, including integration with AP generation and EC coupling. Simulations performed in the study demonstrated links between oscillations of mitochondrial inner membrane potential induced by ROS-induced ROS release, decreased ATP-to-ADP ratio in the cytoplasm, and AP shortening cause by increased ATP-regulated potassium channel current (IKATP) activation. [27, Rank 4]

Metabolic stress that results from myocardial ischemia and reperfusion (I/R) collectively referred to as I/R injury culminates in a wide variety of electromechanical dysfunctions, including lethal arrhythmias such as ventricular tachycardia and ventricular fibrillation (VF). The impact of I/R injury is expected to increase concomitantly with an aging population and the associated increased risk of ischemic heart disease, but efforts to develop a useful therapy to treat I/R injury in the clinical setting have thus far been largely unsuccessful. As ischemia and reperfusion injury stems from pathological changes in many components that exist as part of the highly coupled and nonlinear system of cardiomyocyte physiology, it has been increasingly apparent in recent years that a detailed analysis of multiple pathways is required to sufficiently understand the problem. As such, several groups have developed and used models of cardiomyocyte ischemia and/or reperfusion. Some models have focused on specific components, such as SERCA, L-type calcium channels, and IKATP, whereas others have focused on specific facets of ischemia, including studies of how respiratory acidosis affects development of contractile force and which components play the biggest role in the accumulation of extracellular potassium during ischemia, a common proarrhythmic observance. [28, Rank 2]

Still other efforts have led to the development of more integrated models of I/R pathophysiology. A recent study, which used the first model to fully couple intra- and extracellular pH systems, suggested that inhibition of the Na+-H+ exchanger (NHE) as a strategy to reduce sodium overload during reperfusion may, in fact, be detrimental. Simulations showed that inhibition of intracellular pH recovery resulting from NHE inhibition paradoxically fails to attenuate sodium and calcium overload (despite reduced sodium influx via the NHE), in agreement with at least some experimental observations, and that the failure to attenuate sodium and calcium

overload is caused by inhibition of other cellular components that are sensitive to pH. The ability to monitor many system parameters throughout an entire simulated experiment allowed for insight that cannot be obtained with in vitro and in vivo approaches, where typically only one or two parameters can be observed in a given experiment.

With regard to ischemia and reperfusion injury, there are many interesting unanswered questions. Models could be used to strictly control the degree and rate of calcium accumulation while observing the resulting effects on individual components of the calcium handling system. Another question relates to the utility of suppressing pH recovery during reperfusion, which has been proposed as a strategy to limit reperfusion injury. This has shown to have some benefit in animal experiments, but the jury is still out on whether this is an appropriate strategy given the myriad detrimental effects induced by acidosis. In addition, in one experiment, intracellular pH recovery was only stifled for a short time, presumably other components in the system compensated, allowing intracellular pH to recover. Detailed models in which individual components can see different pH values can answer questions such as what cell components allow intracellular pH recovery in the face of prolonged extracellular acidosis and whether prolonged acidosis is likely to benefit electrical or mechanical function. [29, Rank 5]

It should be noted that there are difficulties associated with developing and using models to address these types of questions, where multiple outputs (e.g., sodium and calcium concentrations, intra- and extracellular pH, ion channel, and pump fluxes) must be observed at the same time. Models must of course be informed by experimental data, so for complex models it is necessary to cull data from a wide variety of sources. Given that data must be used from different laboratories that employ different techniques and reagents, as well as experiments performed sometimes a decade or more apart, tuning and validating models against known behavior for many different parameters is often difficult. In particular, ***modeling changes in ion concentrations is particularly challenging, as sodium, calcium, and potassium are all tightly linked through sodium-calcium and sodium-potassium exchange, both of which are modulated by multiple metabolic changes.*** There are myriad other ways in which a change in the concentration of one ionic species can indirectly affect others. As an example, elevated intracellular sodium can lead to increased calcium load

via reverse sodium-calcium exchange, but elevated calcium will also decrease the inward driving force and facilitate calcium-induced inactivation of L-type calcium channels. Such a plethora of direct and indirect effects makes accurate modeling of multiple ionic species extremely challenging. Developing better models, whether of ischemia or other metabolic syndromes, would be greatly aided by greater consistency in experimental protocols such as those recently described in regard to electrophysiology . Returning to ischemia and reperfusion injury, it would be highly desirable to have a core group of experiments in which sodium, potassium, calcium, pH, phosphometabolite concentrations, membrane voltage, and force development were all measured at the same temperature, using the same buffers, and at the same pacing frequencies. This would require several different experiments of course, but if they were all performed by the same group and, ideally, within a short period of time, many confounding factors that plague model development could be mitigated. [30, Rank 3]

## Conclusion

Neurological dysfunction may affect the control of cardiac rate and rhythm. First, repetitive seizures can induce remodeling of the potassium and Na-channels within the heart, leading to QTc prolongation and cardiac arrhythmias. Second, autonomic neuropathies have previously been described to prolong QTc interval in patients with primary CNS disease, autonomic neuropathy, and amyotrophic lateral sclerosis. The exact mechanism by which altered nervous system control leads to cardiac arrhythmias in these cases is unknown. It has been suspected that sympathovagal imbalance in people with RTT may contribute to sudden cardiac death, even in the presence of normal cardiac function revealed by echocardiography. An important question is whether this imbalance indirectly leads to QTc prolongation. Further characterization in mouse models of RTT may be helpful in understanding the mechanism underlying this prolongation, however, this characterization may be challenging because heart rate is primarily controlled by sympathetic control. [25, Rank 5]

***Important information for post-test is highlighted in red letters, boxes and diagrams.**

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