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Mechanisms of Sudden Cardiac Death: Oxidants and Metabolism

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Abstract

Ventricular arrhythmia is the leading cause of sudden cardiac death (SCD). Deranged cardiac metabolism and abnormal redox state during cardiac diseases foment arrhythmogenic substrates through direct or indirect modulation of cardiac ion channel/transporter function. This review presents current evidence on the mechanisms linking metabolic derangement and excessive oxidative stress to ion channel/transporter dysfunction that predisposes to ventricular arrhythmias and SCD. As conventional anti-arrhythmic agents aiming at ion channels have proven challenging to use, targeting arrhythmogenic metabolic changes and redox imbalance may provide novel therapeutics to treat or prevent life-threatening arrhythmias and SCD.

Keywords

sudden cardiac death; arrhythmias; oxidative stress; metabolism

Introduction

Sudden cardiac death (SCD) refers to death following an unexpected sudden cardiac arrest in a patient with or without known structural heart disease. The incidence of SCD in the United States ranges from 300,000 to 460,000 events per year,^{1,2} depending on the criteria

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Disclosures

Dr. Dudley is an inventor of 13/551,790 A Method for Ameliorating or Preventing Arrhythmic Risk Associated with Cardiomyopathy by Improving Conduction Velocity, 13/507,319 A Method for Modulating or Controlling Connexin43 (Cx43) Level of a Cell and Reducing Arrhythmic Risk, PCT/US2008/011919 Modulation Of Sodium Current by Nicotinamide Adenine Dinucleotide, US 12/929,786 Modulating Mitochondrial Reactive Oxygen Species to Increase Cardiac Sodium Channel Current and Mitigate Sudden Death, and 13/551,790 Method for Ameliorating or Preventing Arrhythmic Risk Associated with Cardiomyopathy.

for SCD used for surveillance. SCD results from a complex interaction between pre-existing cardiac substrates, either structural or genetic, with superimposed physiological or environmental triggers. The most common underlying etiological disorders for SCD in adults age 35 and older are coronary heart disease (CHD, 65–80%)^{2, 3} and dilated cardiomyopathy (DCM, 10–15%).³ Various types of cardiomyopathy (e.g., hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, infiltrative, inflammatory, and valvular diseases), genetically determined rhythm disorders (e.g., long QT syndrome, Brugada syndrome, and catecholaminergic polymorphic ventricular tachycardia) or developmental disorders (anomalous origins of coronary arteries) account for most of the remaining SCDs.^{2, 4} The epidemiology and etiologies of SCD have been extensively reviewed previously⁴ and in the article on “Epidemiology of Sudden Cardiac Death” in this compendium series [Ref].

The physiological mechanisms reported to cause SCD vary with the patient population and the criteria used to define SCD.^{5, 6} In general, ventricular tachyarrhythmias, including ventricular fibrillation (VF) and pulseless ventricular tachycardia (VT), are the most common electrophysiological mechanisms leading to SCD.^{5, 7} Other physiological events that can result in SCD include pulseless electrical activity (PEA), bradyarrhythmias and asystole.^{8, 9} This review will focus only on SCDs attributed to ventricular tachyarrhythmias.

Ventricular tachyarrhythmias, like all cardiac arrhythmias, result from one of three primary mechanisms: reentry, abnormal automaticity, or triggered activity. Reentry is the most common mechanism for ventricular tachyarrhythmias, involving the presence of an anatomical or functional disturbance of cardiac electrical impulse propagation and of heterogeneous conduction. Abnormal automaticity results from the accelerated generation of an action potential by a region of ventricular cells. Triggered activity occurs when an action potential elicits subsequent depolarizations earlier (early afterdepolarization, EAD) or later (delayed afterdepolarization, DAD) in the repolarization phase.

All three mechanisms can result from abnormal functioning of myocardial ion channels and transporters, leading to disordered initiation or propagation of cardiac action potentials. Accumulating evidence suggests that altered cardiac ion channel/transporter function is closely linked to abnormal myocardial metabolic activity and imbalanced redox states in a wide range of cardiac pathology. This review presents the current evidence on the acute effects of abnormal myocardial metabolism and increased oxidative stress on myocardial ion channel/transporters that predispose to ventricular arrhythmias and SCD. It is important, however, to recognize that abnormal metabolism and oxidative stress in non-cardiac myocytes tissues can also contribute to the development of ventricular arrhythmias. For example, altered metabolism and redox state have been implicated in vascular tissue leading to atherosclerosis,^{10–13} which underlies the main substrate for SCD and CHD.^{2, 3} Another example is that abnormal metabolism and increased oxidative stress affect autonomic nervous system, thereby contributing to ventricular arrhythmias and SCD.^{14–17} Within the ventricle, chronic effects of such entities as diabetes and ischemia from atherosclerosis work through mechanisms involving metabolic and oxidative abnormalities to create the substrate of structural heart diseases that leads to SCD. Both the role of aberrant metabolism and oxidants in the chronic creation of such substrate will not be further considered.

Overview of cardiac ionic channels and membrane excitability

Normal functioning of the mammalian heart depends on proper electrical activity involving the initiation of the electrical impulse from pacemaker cells, the propagation of the electrical activity through specialized conduction system and myocardium, and the generation of action potentials in individual myocytes.^{18, 19} A normal cardiac cycle begins with the action potential originating from the cells in sinoatrial node, conducts through the atria, atrioventricular node, His bundle, Purkinje fibers and then spreads throughout the entire ventricular myocardium.²⁰ The proper propagation of cardiac electrical impulse depends on low resistance pathways between cells via gap junctions, which are formed by docking of two connexin hemichannels on appositional sarcolemmal membranes.²¹

Cardiac action potentials are generated through the coordinated activity of various types of ion channels and transporters (Figure 1). Inward current conducting through the voltage-gated Na^+ channel rapidly depolarizes the cell (Phase 0), which is followed by early repolarization (Phase 1) attributed to the transient outward K^+ current (I_{to}). Depolarizing L-type Ca^{2+} currents (I_{CaL}) and multiple repolarizing delayed rectifier K^+ currents (I_{K}) form the plateau phase (Phase 2), which is followed by rapid repolarization (Phase 3) with inactivated I_{CaL} and increasing I_{K} . The repolarization continues with the contribution of inwardly rectifying K^+ current, I_{K1} , until return to the resting membrane potential (Phase 4). The Ca^{2+} influx during the action potential triggers the opening of ryanodine receptor 2 (RyR2) on the sarcoplasmic reticulum (SR), triggering the release of Ca^{2+} from SR into the cytosol. Ca^{2+} then binds to troponin-C of the troponin-tropomyosin complex, resulting in sarcomere longitudinal shortening.

During the diastolic phase, Ca^{2+} is removed from the cytosol through sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA), which pumps cytosolic Ca^{2+} into SR, as well as Na^+ / Ca^{2+} exchanger (NCX), an antiporter membrane protein extruding one Ca^{2+} ion from the myocyte in exchange for three Na^+ ions. Importantly, the maintenance of Na^+ and K^+ gradients across the plasma membrane is mediated by the Na^+/K^+ ATPase, one of the more energy-consuming cellular processes in cardiac myocytes. The energy stored in the resultant electrochemical Na^+ gradient and K^+ gradient provides the driving force for many other transporter/exchangers. Conditions that impair the activity of the aforementioned ion channels/transporters can result in abnormal myocardial electrical functioning, leading to ventricular arrhythmias and SCD.

Myocardial metabolism and energetics

Under physiological conditions, more than 90% of ATP produced in the cardiac myocytes is supplied by mitochondria via oxidative phosphorylation (OXPHOS), and the remainder comes from glycolysis and GTP derived from the tricarboxylic acid (TCA) cycle.²² As the predominant energy generator in the heart, mitochondria occupy ~30% of the volume of cardiac myocytes.²³ The mitochondria have double-membrane structure (inner and outer membranes) that forms separate compartments, the intermembrane space and mitochondrial matrix. Mitochondria metabolize predominantly fatty acids but can metabolize glucose to generate ATP via OXPHOS. Acetyl-CoA, the metabolic intermediate derived from

sequential oxidation of glucose and fatty acid, is utilized to generate the reducing equivalents, nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH₂), in the TCA cycle. NADH and FADH₂ feed electrons to the electron-transport chain (ETC) residing on the mitochondrial inner membrane (Figure 2), where the electrons pass sequentially through complex I, II, III and IV and finally interact with molecular O₂ to form H₂O. Redox reactions occur at complex I, III and IV, driving proton (H⁺) from mitochondrial matrix into the intermembrane space. The resulting proton gradient and strongly negative mitochondrial membrane potential Ψ_m (~–150 to –180 mV)²⁴ help to drive H⁺ flow back to the matrix through mitochondrial ATP synthase (complex V), allowing the conversion of ADP to ATP (Figure 2).

In healthy adult myocardium, 60–90% of acetyl-CoA comes from β -oxidation of fatty acids, whereas 10–40% comes from oxidation of pyruvate derived from glycolysis and lactate oxidation.^{25, 26} While the majority of cardiac ATP is consumed by the myofilaments, it is estimated that ~25% of cardiac ATP hydrolysis is used to fuel sarcolemmal and SR ion channels and transporters.²⁷ The critical dependence of cardiac ion channels and transporters on energy supply from metabolized carbon fuels becomes evident under conditions such as myocardial ischemia, during which the mismatch between ATP supply and utilization can readily disrupt the cardiac rhythm through depleting energy supply to these channels and transporters.^{28, 29}

Cardiac oxidants and oxidative stress

In biological systems, partially reduced forms of oxygen (O₂) lead to the formation of “oxidants” or “reactive oxygen species (ROS)”, which oxidize other molecules when these molecules accept electrons. The main ROS in cardiac myocytes exist in radical forms such as superoxide (O₂^{•–}) and hydroxyl radicals (•OH) or in non-radical forms such as hydrogen peroxide (H₂O₂), hypochlorite (HOCl), nitric oxide (NO•) and peroxynitrite (ONOO[–]). It is important to note that although grouped under the acronym of “ROS”, different reactive species containing molecular oxygen vary greatly in diffusion coefficient, reactivity and oxidization potential.

Under physiological conditions, trace amounts of “signaling ROS”, which are tightly regulated by the balance of ROS production and ROS scavengers, form a network that coordinates metabolism with gene transcription and enzymatic activity.^{30, 31} Low levels of ROS produced during short periods of ischemia, for example, trigger signaling pathways that confer cardiac protection, a phenomenon coined “ischemic preconditioning (IPC)”, which is mediated, at least in part, through mitochondrial K_{ATP} channels (see below).³² In addition, low levels of NADPH oxidase 2 (NOX2)-mediated ROS production sensitizes nearby RyRs and triggers Ca²⁺ sparks in cardiac myocytes in response to physiological stretch with increased preload³³ and afterload,³⁴ a process termed X-ROS signaling. Excessive ROS leads to detrimental reactions with cellular lipids and proteins, eliciting maladaptive responses and other abnormalities that compromise cellular and tissue functions, ultimately leading to cell death.^{35–37} The deleterious effects caused by excessive ROS are defined as oxidative stress.

The main sources of ROS in cardiac myocytes include nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, xanthine oxidase, nitric oxide synthase (NOS) and mitochondria.^{38, 39} Figure 3 illustrates the major ROS synthesis pathways in cardiac myocytes. NADPH oxidases are membrane-bound enzymes that use NADH or NADPH as the electron donor to generate $O_2^{\bullet-}$ from O_2 .⁴⁰ Xanthine oxidase catalyzes the oxidation of hypoxanthine/xanthine and produces $O_2^{\bullet-}$.⁴¹ NOS, on the other hand, produces a single nitrogen radical ($NO^{\bullet}$) through metabolizing L-arginine to L-citrulline. NOS becomes uncoupled under conditions such as depletion of L-arginine, oxidation of the NOS cofactor tetrahydrobiopterin (BH_4), or increased S-glutathionylation of NOS. Under these conditions NOS produces $O_2^{\bullet-}$ instead of $NO^{\bullet}$.^{42, 43} $NO^{\bullet}$ itself is a weak thiol oxidant, but it regulates various physiological functions by activating cGMP/protein kinase G (PKG) pathways or by covalent modification of protein cysteine residues via S-nitrosylation (SNO) or S-glutathiolation (GSS-);^{44, 45} $NO^{\bullet}$ also reacts with $O_2^{\bullet-}$ and forms a more potent oxidative molecule peroxynitrite ($ONOO^-$). Oxidative molecules derived from $NO^{\bullet}$, such as $NO_2^{\bullet}$, N_2O_3 and $ONOO^-$, are termed reactive nitrogen species (RNS).^{44, 45} As the metabolic hub in cardiac myocytes, mitochondria consume most of the O_2 in cardiac myocytes for energy production, thereby serving as the major source of ROS in the heart.

In the mitochondria, ROS are generated as an inevitable byproduct of OXPHOS, resulting from the premature leak of electrons from the ETC to molecular oxygen. It has been estimated that 0.1–1% of the electrons passing through the ETC prematurely leak to O_2 at complexes I, II or III, leading to $O_2^{\bullet-}$ formation (Figure 2).⁴⁶ The ROS production rate in the mitochondria is accelerated by increased proton motive force, by the increased NADH/ NAD^+ ratio, by the increased ratio of reduced coenzyme Q10 (CoQH2) to coenzyme Q10 (CoQ), or when the O_2 concentration is raised above physiological levels.^{47, 48} Paradoxically, mitochondrial ROS production also increases under condition of very low local O_2 concentration.^{47, 49} Mitochondrial $O_2^{\bullet-}$ is converted to hydrogen peroxide (H_2O_2) and O_2 by manganese superoxide dismutase (MnSOD), the primary antioxidant enzyme in mitochondria.⁵⁰ H_2O_2 is then converted to H_2O and O_2 by cytosolic and mitochondrial scavenging enzymes, glutathione peroxidases, peroxiredoxins and catalase. The emission of mitochondrial ROS is determined by the tight balance between the redox-dependent ROS production and elimination. The concept of “redox-optimized redox balance” implies that a reduced mitochondrial redox state (high NADH) not only favors mitochondrial ROS production, but at the same time optimizes ROS elimination through NADPH-dependent ROS scavenging system such as glutathione (GSH) and thioredoxin.⁵¹ Cardiac mitochondria are optimized to keep ROS emission at minimum by operating at an intermediate redox state, where ROS emission is highest under very oxidized or very reduced conditions.^{51, 52} During conditions with marked oxidation (such as heart failure), depletion of the antioxidative capacity leads to excessive ROS outflow in spite of reduced $O_2^{\bullet-}$ production from the ETC, whereas during highly reduced conditions (such as ischemia), excessive $O_2^{\bullet-}$ production from the ETC overwhelms the antioxidative capacity, in spite of the abundant NADPH levels.^{51–53}

The mitochondrial ETC efficiency is impaired under pathological conditions such as myocardial ischemia^{54, 55} and heart failure,^{56, 57} resulting in increased electron leak and ROS production. Accumulating ROS levels trigger the opening of mitochondrial channels

including mitochondrial permeability transition pore (PTP)⁵⁸ and inner membrane anion channel (IMAC) (Figure 2),⁵⁹ leading to depolarization of the Ψ_m and further acceleration of ROS production, a phenomenon termed “mitochondrial ROS-induced ROS release.”^{60–62} The PTP is a non-selective channel residing on the inner mitochondrial membrane, and it opens with elevated matrix $[Ca^{2+}]$, increased ROS and phosphate levels, as well as by reduced adenine nucleotides (ADP or ATP).⁶³ Brief and reversible PTP opening is considered physiological and serves as a release mechanism to prevent overt mitochondrial cation (especially Ca^{2+}) and ROS accumulation.⁶⁴ During myocardial ischemia, myocytes experience Ca^{2+} overload, high phosphate and low ATP concentrations. These conditions prime PTP for opening when reperfusion triggers ROS generation and mitochondrial Ca^{2+} accumulation, leading to prolonged PTP opening and burst ROS release from mitochondria.^{63, 64} Pharmacological inhibition of PTP (with cyclosporine A or sanglifehrin A) provides protection against ischemia-reperfusion injury in various experimental models.^{65, 66} Recent evidence suggests that dimers of mitochondrial ATP-synthase form the pore-forming component of PTP,⁶⁷ and the c subunit of the ATP-synthase complex may be required for PTP-dependent mitochondrial fragmentation and cell death.⁶⁸ IMAC, on the other hand, is responsible for anionic currents across the mitochondrial membrane, allowing the passage of $O_2^{\bullet-}$ generated by ETC from the matrix to the cytoplasmic side of the inner membrane, thereby serving as a mitochondrial $O_2^{\bullet-}$ efflux and Ψ_m depolarization mechanism.⁶⁹ During ischemia and reperfusion, mitochondrial $O_2^{\bullet-}$ production is increased, which leads to increased permeability of IMAC, resulting in a burst in ROS release and Ψ_m depolarization. The resulting reversible Ψ_m collapse and membrane potential oscillation foster lethal ventricular arrhythmias, which can be abrogated by IMAC inhibitors.⁶⁹ During mitochondrial ROS-induced ROS release, depolarized mitochondrial Ψ_m depletes NADPH (the level of which is coupled to [NADH] by nicotinamide nucleotide transhydrogenase in mitochondria), thereby leading to rapid dissipation of ROS-eliminating capacity, allowing acceleration of ROS emission from mitochondria.^{51, 60–62}

Elevated mitochondrial ROS also impairs ATP production through various mechanisms. Excessive ROS cause oxidative damage to components of the ETC such as complex I, II,⁷⁰ IV⁷¹ and ATP synthase,⁷⁰ impairing ATP generation and further aggravate electron leakage. In addition, $O_2^{\bullet-}$ activates mitochondrial uncoupling proteins, resulting in increased electron leak and uncoupled O_2 consumption from ATP synthesis.^{72, 73} Taken together, excessive cardiac ROS leads to abnormal electrical function directly by ROS-mediated signaling and oxidative damage, as well as indirectly by decreasing ATP generation that is required for normal ion channel/transporters functioning.^{28, 29}

Impaired cardiac metabolism and increased myocardial oxidative stress, depending on the etiology and clinical course, can be divided into two categories: acute and chronic. Acute cardiac oxidative and metabolic derangements typically occur with myocardial ischemia/reperfusion that results from coronary artery occlusion. Chronic oxidative and metabolic abnormalities, in contrast, occur with conditions such as cardiac hypertrophy, heart failure and diabetes mellitus. The impact of chronic oxidative and metabolic stresses on cardiac electrophysiology often involves changes in the transcript and protein expression of cardiac ion channels/transporters, which is commonly defined as “electrical remodeling.”⁷⁴ Electrical remodeling during these chronic cardiac conditions have been extensively

reviewed elsewhere.^{74, 75} Aside from a few exceptions, therefore, this review will focus on the effects of acute myocardial metabolic and oxidative stress on cardiac electrophysiology.

Mechanisms linking impaired cardiac metabolism to ventricular arrhythmias and SCD

A. Metabolic regulation of cardiac Ca^{2+} handling and homeostasis

Calcium ions are critical intracellular signaling molecules, responsible for the regulation of numerous cellular processes in cardiac myocytes including excitation-contraction coupling, gene transcription, enzyme activity and cell death.⁷⁶ The intracellular $[\text{Ca}^{2+}]$ concentration fluctuates markedly between systole and diastole, yet the changes in cytosolic $[\text{Ca}^{2+}]$ are tightly regulated. Multiple signaling molecules, including calcium/calmodulin-dependent protein kinase II (CaMKII), protein kinase A (PKA) and protein kinase C (PKC), are involved in the regulation of these Ca^{2+} handling proteins (see review by Bers).⁷⁶ Impaired Ca^{2+} homeostasis and handling have been implicated in the mechanical dysfunction and arrhythmogenesis observed in acute myocardial ischemia,^{77, 78} as well as in chronic cardiac conditions such as cardiac hypertrophy^{79, 80} and heart failure.^{81, 82}

During myocardial ischemia or metabolic inhibition, various metabolic parameters are markedly altered in cardiac myocytes. For example, ATP levels decrease, cells become progressively acidic with elevated lactate levels,⁸³ and the levels of phosphate⁸⁴ and magnesium⁸⁵ increase. Both reduced ATP and elevated phosphate levels can inhibit Na^+/K^+ ATPase activity,^{86, 87} leading to intracellular Na^+ accumulation. Increased late Na^+ -currents (late I_{Na}) also contributes to increased intracellular $[\text{Na}^+]$ during myocardial ischemia^{88–90} and heart failure.⁹¹ Elevated intracellular $[\text{Na}^+]$ leads to increased cytosolic $[\text{Ca}^{2+}]$, at least in part, through decreased extrusion of Ca^{2+} or through actual Ca^{2+} entry with NCX activity in the reverse mode (Na^+ out and Ca^{2+} in).^{92–94} The activity of SR Ca^{2+} uptake (through SERCA) and release (through RyR2) are both inhibited during myocardial ischemia;²⁸ however, RyR2 inhibition appears to predominate over reduced SERCA activity during ischemia, reflected in lower frequency of spontaneous Ca^{2+} release through RyR2^{28, 95} and increased SR Ca^{2+} load.²⁸ Upon reperfusion or re-oxygenation, RyR2 is released from inhibition, producing spontaneous waves of Ca^{2+} release,⁹⁶ which may contribute to calcium transient/action potential alternans and increase the risk of ventricular arrhythmias (Figure 4 and Table 1).^{97, 98}

In addition to the ATP production machinery, mitochondria also harbor Ca^{2+} channels and transporters, allowing the transfer of Ca^{2+} ions between cytosol and mitochondria, thereby contributing to the dynamic regulation of sarcolemmal Ca^{2+} homeostasis. Mitochondria uptake Ca^{2+} predominantly through the mitochondrial Ca^{2+} uniporter (MCU) and extrude Ca^{2+} mainly through the mitochondrial $\text{Na}^+-\text{Ca}^{2+}$ exchanger (mitoNCX) (Figure 2).^{99, 100} The capacity of Ca^{2+} uptake and release by mitochondria forms an additional buffer for cytosolic Ca^{2+} regulation, contributing to the spatiotemporal dynamics of Ca^{2+} signaling in cardiac cells.^{101, 102} Nevertheless, it remains debatable how much mitochondria contribute to cellular Ca^{2+} dynamics under physiological and pathological conditions.¹⁰³ Recently Williams et al.¹⁰² provide quantitative data suggesting that mitochondria do not function as

a significant buffer of cytosolic Ca^{2+} under physiological conditions; with prolonged elevation of cytosolic $[\text{Ca}^{2+}]$, however, mitochondrial Ca^{2+} uptake can increase up to 1000-fold and impact cellular Ca^{2+} dynamics significantly.¹⁰² Nevertheless, it has been shown that mitochondrial dysfunction results in cardiac myocytes Ca^{2+} transient alternans by impairing the mitochondrial capacity of Ca^{2+} handling, thereby predisposing to cardiac arrhythmias.¹⁰⁴ In addition, pharmacological inhibition of mitochondrial uptake through MCU with Ru360 has been shown to reduce the incidence of ventricular arrhythmias induced by ischemia-reperfusion in the rodent heart.¹⁰⁵ As the metabolic center in cardiac myocytes, therefore, mitochondria play an important role in transducing changes in cardiac metabolic states to the dynamic regulation of sarcolemmal Ca^{2+} , membrane excitability and electrical functioning.

B. Metabolic regulation of Na^+ homeostasis and Na^+ channel function

Intracellular $[\text{Na}^+]$ plays a critical role in regulating the energetics, electrical functioning and contractility of cardiac myocytes, which can be attributed to the direct and indirect effects of cytosolic $[\text{Na}^+]$ on Ca^{2+} homeostasis,⁹² mitochondrial function,¹⁰⁶ and cellular signaling.¹⁰⁷ Multiple ion channels/transporters function to maintain the $[\text{Na}^+]$ homeostasis in cardiac myocytes: Na^+/K^+ ATPase, for example, consumes ATP to pump Na^+ outside of the cell in exchange of K^+ , representing the main mechanism of Na^+ efflux, whereas Na^+ channels, NCX and the Na^+/H^+ exchanger (NHE) mediate Na^+ influx in cardiac myocytes.

Cardiac voltage-gated Na^+ channels form by the assembly of a pore-forming α subunit and auxiliary β subunits that modulate channel activities. Nav1.5 (*SCN5A*) is the predominant Nav α subunit expressed in the mammalian myocardium. Currents conducting through Na^+ channels generate the rapid upstroke (phase 0) of the action potential. In addition, Na^+ channels, together with cardiac gap junctions, govern the electrical conduction velocity in the myocardium. There are two effects of Na^+ channel/transporter dysfunction on arrhythmogenesis: (1) through affecting $\text{Na}^+/\text{Ca}^{2+}$ homeostasis, thereby promoting delayed afterdepolarizations (DADs) and creating reentry substrates, and (2) through electrophysiological effects of the channel function. For example, increased late I_{Na} predisposes to APD prolongation and EADs, both of which are arrhythmogenic and predispose to SCD (Table 1 and Figure 4).

During the metabolic stress that ensues with myocardial ischemia/reperfusion, reduced Na^+/K^+ ATPase (because of reduced ATP and elevated ADP/phosphate levels), increased late I_{Na} (see below for detailed mechanisms)¹⁰⁸ and enhanced NHE (owing to increased cytosolic acidosis) activity^{109, 110} result in increased cytosolic $[\text{Na}^+]$, thereby leading to Ca^{2+} overload and increased propensity for ventricular arrhythmias (see above). It has been shown that pharmacological inhibition or knockout of NHE renders cardiac function resistant to ischemia-reperfusion injury.¹¹¹

In addition to altered Na^+/K^+ ATPase and NHE function, it has been demonstrated that ischemia/metabolic inhibition can trigger Na^+ influx through connexin (Cx) hemichannels.^{112, 113} Under normal conditions Cx hemichannels located on adjacent intercellular sarcolemmal membranes combine to form gap junctions at the intercalated discs between cardiac myocytes. During ischemia/metabolic inhibition, Cx hemichannels

localize to the nonjunctional membrane, turning into non-selective cation channels that are permeable to Na^+ , K^+ and Ca^{2+} .^{112, 114} With the high conductance of these Cx hemichannels, it is suggested that the opening of even a small number of Cx43 hemichannels can lead to doubling of Na^+ influx in ventricular cardiac myocytes,^{114, 115} contributing to increased Na^+ load and increased propensity for arrhythmias (Table 1 and Figure 4).

Elevated cytosolic $[\text{Na}^+]$ also impairs cardiac metabolism reciprocally through affecting mitochondrial function. Mitochondrial $[\text{Na}^+]$ is regulated by NHE-mediated Na^+ uptake¹¹⁶ and mitoNCX-mediated Na^+ efflux.¹¹⁷ Under physiological conditions, energized mitochondria extrude H^+ , and the resulting pH gradient drives the Na^+ gradient between mitochondrial matrix (lower $[\text{Na}^+]$) and cytosol (higher $[\text{Na}^+]$).^{116, 118} Intracellular Na^+ levels increase significantly in pathological conditions such as myocardial ischemia.¹⁰⁹ The rise in cytosolic $[\text{Na}^+]$ during ischemia widens the Na^+ gradient across mitochondria, leading to stronger driving force for Ca^{2+} extrusion from mitochondria via mitoNCX, thereby leading to reduced mitochondrial $[\text{Ca}^{2+}]$ and altered mitochondrial energetics,^{100, 106} including decreased activities of Ca^{2+} -dependent dehydrogenases in the TCA cycle, the net oxidation of the matrix NADH/NADPH pool, and hence the deficiency of NADPH-dependent ROS scavengers, thereby increasing mitochondrial ROS emission.^{119, 120} Increased ROS levels further aggravate cytosolic Na^+ accumulation by increasing late I_{Na} through direct Na^+ channel modification^{121, 122} or by activating signaling molecules such as PKC¹²³ or CaMKII (see below).¹²⁴ Therefore, there may be a feed-forward mechanism between increased cytosolic $[\text{Na}^+]$, impaired mitochondrial metabolism, increased ROS emission, and ROS-induced elevation of cytosolic $[\text{Na}^+]$.¹²⁵

In addition to cytosolic $[\text{Na}^+]$, cardiac metabolism also influences electrical functioning through modulating sarcolemmal Na^+ channel activity.¹²⁶ During myocardial ischemia, various metabolic derangements result in altered Na^+ channel properties and function. The ischemic metabolite lysophosphatidylcholine (LPC), for example, has been shown to decrease peak Na^+ current (peak I_{Na}) and to slow its inactivation.¹²⁷ In addition, LPC also causes a marked increase in late I_{Na} , thereby increasing myocardial Na^+ loading.⁸⁸ Myocardial acidosis during ischemia is also known to affect the inactivation states of Na^+ channel, leading to increased late I_{Na} (Table 1 and Figure 4).^{89, 90} Cardiac Na^+ channels are reported to be modulated by adenosine monophosphate-activated protein kinase (AMPK). AMPK is a serine/threonine kinase that is activated by increased cytosolic AMP/ATP ratio to increase ATP production and decrease ATP consumption, thereby functioning as a master metabolic regulator in cardiac myocytes.^{128, 129} Mutations in PRKAG2 gene, which encodes the $\gamma 2$ subunit of AMPK, have been reported to be associated with electrical instability and lethal ventricular arrhythmias.^{130–132} For example, PRKAG2 T172D, a constitutively active AMPK mutant, has been shown to slow Na^+ channels inactivation, leading to increased late I_{Na} , prolonged APD and arrhythmogenic EADs.¹³⁰

C. Metabolic regulation of K^+ channels and cardiac repolarization

Various types of voltage-gated K^+ (Kv) channels and non-voltage-gated inwardly rectifying (Kir) channels contribute to myocardial action potential repolarization.^{19, 20} Under

pathological conditions such as myocardial ischemia, diabetes and heart failure, repolarizing Kv currents in ventricular cardiac myocytes are reduced, leading to delayed repolarization and prolonged APD, which predispose to the development of ventricular arrhythmias and SCD (Figure 4).²⁰

Among several types of Kir channels functioning in mammalian heart, I_{K1} contributes to the terminal phase of repolarization and the maintenance of resting membrane potentials in ventricular myocytes,^{20, 133, 134} whereas currents conducted via sarcolemmal K_{ATP} (sarcK_{ATP}) channels ($I_{K_{ATP}}$), gated by intracellular ATP/ADP levels and acidosis,¹³⁵ function as immediate sensors of cellular metabolism and play an essential role in electrophysiological responses to metabolic stresses such as myocardial ischemia.^{136, 137}

Abnormal cardiac repolarization, including QT-interval prolongation and T wave abnormalities, are often observed in patients with diabetes, one of the leading chronic metabolic disorders associated with ventricular arrhythmias and sudden cardiac death.^{138, 139} Studies on cellular mechanisms of diabetes-induced repolarization abnormalities have consistently revealed downregulation of cardiac Kv currents in diabetic heart.^{140–142} Multiple mechanisms have been proposed to account for reduced Kv currents during diabetes. First, post-translational inhibition of Kv channel function occurs in diabetes. Plasma levels of free fatty acid metabolites, such as palmitoylcarnitine and palmitoyl-CoA, are increased with diabetes. They have been shown to inhibit cardiac Kv currents directly.¹⁴³ Acute treatment with insulin has been demonstrated to reverse diabetes-induced Kv current reduction, suggesting the primary role of insulin-dependent signaling on Kv channel function.¹⁴⁰ It has been suggested that improved glucose utilization by insulin may be responsible for restored Kv current expression, as compounds known to improve glucose utilization (such as dichloroacetate or L-carnitine) have been shown to normalize cardiac Kv current density with diabetes.¹⁴¹ Increased ROS levels also contribute to reduced cardiac Kv currents during diabetes, and direct incubation with antioxidant GSH has been shown to restore Kv current expression in myocytes isolated from diabetic hearts.¹⁴⁰ Second, transcriptional regulation of Kv channel expression may reduce Kv currents. Peroxisome proliferator-activated receptor α (PPAR α), a critical regulator of glucose/fatty acid metabolism, is upregulated in diabetic heart and has been suggested to transcriptionally repress cardiac Kv channel expression.¹⁴⁴ Cardiac-specific over-expression of PPAR α downregulates the mRNA and protein expression levels of Kv4.2 and KChIP2, the channel subunits encoding I_{to} ,¹⁴⁴ whereas PPAR α knock-out upregulates I_{to} .¹⁴⁴ Interestingly, it has been recently demonstrated that the activation of phosphoinositide 3-kinase α (PI3K α), a key component of insulin receptor signaling pathway that can be triggered by insulin treatment or exercise training, upregulates mRNA levels of various Kv channels^{145, 146} through an Akt-independent mechanism,¹⁴⁷ which may provide the mechanistic explanation for insulin-mediated Kv current modulation, as well as the metabolic impact of exercise training on cardiac repolarization. It is important to note that the changes in Kv current observed with diabetes or exercise training reflect the chronic effects of metabolic stress on Kv channel remodeling, rather than acute channel effects.

The sarcK_{ATP} channels are present at high density in the sarcolemmal membrane. SarcK_{ATP} channels are inhibited by ATP and activated by ADP, Mg^{2+} or low pH, conditions that are

associated with ischemia, insufficient fuel supply, and increased metabolic stress.¹⁴⁸ Upon increased metabolic stress with ATP depletion and acidosis (e.g., during myocardial ischemia),¹⁴⁹ sarcK_{ATP} channels are activated, allowing an inwardly rectifying repolarizing K⁺ current. SarcK_{ATP} channels open within seconds in response to acute ischemia, which is likely attributed to the rapid drop in pH (also within seconds of ischemia) in the ischemic tissue.¹⁵⁰ ATP levels, by contrast, deplete at a slower rate and remain in the millimolar range (levels that are sufficient to keep sarcK_{ATP} channels closed) until 10–15 minutes after the onset of ischemia.¹⁵¹ Therefore, ATP depletion may not contribute to sarcK_{ATP} channel opening during the early phase of ischemia.

The cell surface expression density of sarcK_{ATP} channels is very high. It has been estimated that the opening of merely 1% of the sarcK_{ATP} channels is sufficient to significantly shorten myocardial APD.¹⁵² The opening of sarcK_{ATP} channels shortens APD and decreases inward Ca²⁺ currents, thereby reducing Ca²⁺-mediated cardiac energy consumption and preventing Ca²⁺ overload-induced cell death. With adequate numbers of sarcK_{ATP} channels in the open state, however, cardiac myocytes become hyperpolarized and rendered unexcitable.¹⁵³ This creates a “current sink” that slows or blocks electrical propagation in the myocardium, predisposing to the development of ventricular arrhythmia (Table 1 and Figure 4).^{62, 154} Indeed, pharmacological inhibition of sarcK_{ATP} channels has been shown to reduce the incidence of ventricular arrhythmias in animal models^{155–157} and in humans.^{158–160} Reduced mitochondrial ATP production with impaired respiratory chain function leads to activation of sarcK_{ATP} channels. During ROS-induced ROS release, for example, dissipation of Ψ_m results in cessation of mitochondrial ATP production, leading to sarcK_{ATP} activation, altered myocyte APDs and even ventricular arrhythmias.^{59, 62, 161} Interestingly, it has been shown that ATP generated from glycolysis plays a bigger role in modulating sarcK_{ATP} activity than ATP generated from OXPHOS,¹⁶² which is in accordance with recent findings that the key glycolytic enzymes, including glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and pyruvate kinase, serve as components of the sarcK_{ATP} channel macrocomplex and regulate its function.^{163, 164} SarcK_{ATP} channels have also been shown to exhibit reduced sensitivity to ATP-mediated inhibition in diabetic heart, resulting in higher sarcK_{ATP} conductance and greater APD shortening in response to hypoxia.¹⁶⁵

D. Non-ion channel mechanisms linking metabolism to ventricular arrhythmias and SCD

As mentioned above, AMPK functions as a key metabolic sensor and regulator in cardiac myocytes. It has been reported that specific mutations in PRKAG2, the γ 2 regulatory subunit of AMPK, lead to a glycogen storage cardiomyopathy characterized by ventricular pre-excitation and cardiac hypertrophy.^{166, 167} The structural abnormalities associated with PRKAG2 mutations, including the glycogen accumulation and ventricular hypertrophy, result in abnormal conduction that predisposes to ventricular arrhythmias.^{166, 167} In addition to AMPK, patients with inherited fatty acid oxidation disorders exhibit high incidence of ventricular arrhythmias, conduction defects and SCD,¹⁶⁸ which can be attributed to the accumulation of arrhythmogenic intermediary metabolites of fatty acids.¹⁶⁸ Fatty acid metabolites such as LPC accumulate during myocardial ischemia, contributing to the formation of arrhythmogenic substrates.⁸⁸ Consistent with these findings, a transgenic

mouse model of cardiac-specific overexpression of fatty acid transport protein 1 (α -MHC FATP1) has been shown to exhibit increased myocardial uptake and accumulation of free fatty acids, leading to arrhythmogenic electrical changes including QT prolongation.¹⁶⁹ Taken together, these data suggest that abnormal cardiac metabolism can lead to ventricular arrhythmias and SCD through non-ion channel mechanisms.

Mechanisms linking increased cardiac oxidative stress to ventricular arrhythmias and SCD

A. Myocardial oxidative stress and cardiac Na^+ channels

Abnormal cardiac Na^+ channel function is often observed in cardiac diseases such as myocardial ischemia^{170, 171} and heart failure,^{91, 172} conditions that are associated with increased cardiac oxidative stress. Na^+ channel abnormalities can be categorized as loss- or gain-of function. Loss-of-function Na^+ channel abnormality leads to reduced peak I_{Na} , resulting in conduction block that potentiates re-entrant type ventricular arrhythmias.¹⁷³ Gain-of-function Na^+ channel abnormality, in contrast, results in the increase in late I_{Na} , which can lead to prolongation of APDs, EADs, reverse mode NCX transport and subsequent Ca^{2+} overload in sarcomere, all of which predispose to ventricular arrhythmias.^{122, 174}

ROS have been demonstrated to affect Na^+ channel function through multiple mechanisms. Transcriptionally, ROS have been shown to reduce Nav1.5 channel expression by reducing mRNA expression.^{175, 176} At the protein level, ROS have been shown to impair Nav1.5 channel inactivation through direct oxidation at the methionine residues of Nav1.5 channel protein.¹⁷⁷ Elevated mitochondrial ROS are known to reduce peak I_{Na} by modifying Nav1.5 conductance through post-translational mechanisms without affecting cell surface expression of Nav1.5.⁵⁷ The slowly inactivating component of Na^+ current (late I_{Na}), on the other hand, is known to be augmented by ROS in cardiac myocytes.^{121, 122, 174} For example, increased H_2O_2 has been shown to increase the open probabilities of Na^+ channel, leading to increased late I_{Na} ,^{121, 122} and arrhythmogenic changes including prolongation of APDs, EADs, and cytosolic Ca^{2+} overload (Figure 5 and Table 2).^{122, 174} In addition to direct ROS-dependent effects on Na^+ channels, ROS also modulate Na^+ channel activities indirectly by altering membrane lipid environment¹⁷⁸ or by activating signaling molecules such as PKC¹²³ or CaMKII.¹²⁴ Elevated mitochondrial ROS activate PKC, for example, which may reduce peak I_{Na} by affecting Nav1.5 phosphorylation. ROS-induced CaMKII δ activation, on the other hand, has been shown to mediate H_2O_2 -induced increases in late I_{Na} .¹²⁴

Increased myocardial oxidative stress is often associated with impaired cardiac metabolism. The cardiac NADH level, for example, is elevated during cardiac ischemia and cardiomyopathy, which leads to increased mitochondrial ROS production and reduced peak I_{Na} . Using a mouse model of nonischemic cardiomyopathy, we have demonstrated recently that the cytosolic NADH and mitochondrial ROS levels are increased with cardiomyopathy, resulting in a cardiac peak I_{Na} reduction without altering membrane Na^+ channel protein expression levels.⁵⁷ The reduced peak I_{Na} can be restored by mitochondrial antioxidants

NAD⁺ or Mito-TEMPO treatment.⁵⁷ Consistent with these findings, NAD⁺ treatment has been shown to improve myocardial conduction abnormality that is associated with reduced peak I_{Na} in human failing heart.⁵⁷

The link between mitochondrial ROS and I_{Na} regulation is also observed in the hereditary arrhythmia syndromes. One of the Brugada syndrome-causing mutations, A280V mutation in glycerol-3-phosphate dehydrogenase 1-like (GPD1-L) protein, has been demonstrated to reduce peak I_{Na} by increasing cytosolic NADH levels and mitochondrial ROS.^{123, 179} Peak I_{Na} reduction by A280V GPD1-L is abrogated by the treatment of NAD⁺ or Mito-TEMPO.¹²³ Taken together, these data suggest the important role of mitochondrial ROS in transducing the altered cardiac metabolic states (NADH/NAD⁺ levels) to the regulation of cardiac Na⁺ channels and membrane excitability.

During acute ischemia, myocardial NO[•] production is enhanced owing to increased NOS (especially eNOS) activity¹⁸⁰ and conversion of tissue nitrate to NO[•].¹⁸¹ Increased NO[•] production and elevated NOS activities play a critical role in the cardioprotective effects of IPC (see below Section C), which has been extensively reviewed previously.^{182, 183} Elevated NO[•] has been shown to increase late I_{Na},¹⁸⁴ which can be abolished by reducing agents GSH and DTT,^{184, 185} suggesting that NO[•] modulates Na⁺ channel activities by oxidizing Na⁺ channel or its regulatory proteins. Interestingly, others report that NO[•] decreases peak I_{Na} through reducing the open probability and the surface expression of functional Na⁺ channels, which are mediated indirectly through a PKG/PKA-dependent pathway.¹⁸⁶ Peroxynitrite, formed by NO[•] and O₂^{•-} interaction, has also been shown to augment late I_{Na}.¹⁸⁷

B. Cardiac redox state and Ca²⁺ homeostasis

As mentioned earlier in this review, intracellular [Ca²⁺] is tightly regulated by various Ca²⁺ handling proteins, including voltage-gated Ca²⁺ channels, SERCA, RyR2, NCX and signaling molecules such as CaMKII, PKA and PKC. Many of these Ca²⁺ handling and regulatory proteins harbor methionines or thiol groups that are susceptible to the direct modification by ROS or reducing agents, thereby sensitive to the altered redox states during cardiac diseases.

In general, elevated ROS levels in cardiac myocytes are known to result in a net increase in intracellular [Ca²⁺].¹⁸⁸ The direct application of H₂O₂ or increased mitochondrial ROS has been shown to increase I_{CaL} and its sensitivity to isoproterenol stimulation in ventricular myocytes.¹⁸⁹ In addition, oxidized LDL has been demonstrated to stimulate I_{CaL} through LPC-induced mitochondrial ROS.¹⁹⁰ Nevertheless, there are conflicting reports showing reduced cardiac I_{CaL} in the presence of elevated oxidative stress.^{191, 192} The discrepant findings in the effects of ROS on cardiac I_{CaL} may be explained by the differences in the experimental design, animal species and type of ROS involved.

The results on the impact of increased oxidative stress on other Ca²⁺ handling proteins, in contrast, are more consistent. Excessive ROS increases the open probability of RyR2, enhancing the release of Ca²⁺ from SR.¹⁹³ Calcium sparks from RyR2 are increased in response to photoactivated or chemical-induced mitochondrial ROS.¹⁹⁴ In contrast to RyR2,

SERCA activity is reduced upon increased cardiac ROS,^{195–197} which can be, at least in part, attributed to reduced ATP supply for SERCA pump owing to mitochondrial dysfunction.³⁸ Cardiac NCX, which normally extrudes Ca^{2+} from cytosol in exchange for Na^+ , has been shown to be activated in the reverse mode by increased oxidative stress, thereby increasing cytosolic $[\text{Ca}^{2+}]$ (Table 2, and Figure 5).^{198, 199}

CaMKII is activated by ROS, and it is known to play a critical role in regulating calcium handling proteins in response to increased cardiac oxidative stress.²⁰⁰ For example, activated CaMKII augments I_{CaL} by phosphorylating Cav1.2 channel subunit and increasing its open probability.²⁰¹ Phosphorylation of RyR2 by CaMKII increases SR Ca^{2+} leak, promoting cytosolic Ca^{2+} overload and DADs.²⁰⁰ Taken together, the net impact of increased oxidative stress on Ca^{2+} handling proteins leads to cytosolic Ca^{2+} overload and depletion of SR Ca^{2+} store, resulting in detrimental changes such as arrhythmogenic DADs and contractile dysfunction.

As discussed earlier in this review, mitochondria also play an important role in the regulation of Ca^{2+} in cardiac myocytes. Increased oxidative stress is known to modulate mitochondrial $[\text{Ca}^{2+}]$ by altering mitochondrial ion channel activities, contributing to the perturbation of cytosolic Ca^{2+} homeostasis. For example, increased ROS during myocardial ischemia depolarize Ψ_m ,²⁰² forcing mitochondrial NCX into reverse mode and drives Ca^{2+} from cytosol into mitochondria.^{117, 203} Physiologically, mitochondrial Ca^{2+} is required for normal ETC function and serves as a positive effector of OXPHOS. Ca^{2+} overload, however, leads to increased mitochondrial ROS production through mechanisms including increased electron leakage,^{204, 205} enhanced NO production, which is known to inhibit complex IV and augment ROS production from complex III,²⁰⁶ and reduced cytochrome c-mediated respiration.^{207, 208} In addition, pathological mitochondrial Ca^{2+} overload has been shown to increase NO and ROS production in cardiac myocytes.²⁰⁹ These data suggest a positive feedback loop between ROS-induced Ca^{2+} -overload and Ca^{2+} -induced ROS production. Under pathological conditions such as myocardial ischemia, cellular and mitochondrial $[\text{Ca}^{2+}]$ increase, leading to increased ROS production. ROS over-production induced by elevated mitochondrial $[\text{Ca}^{2+}]$ results in further increase in mitochondrial Ca^{2+} and ROS levels. The Ca^{2+} load and ROS produced from this positive feed-forward loop can overwhelm the cellular capacity of ROS scavenging and Ca^{2+} clearance, resulting in cellular damage and electrical instability that predispose to ventricular arrhythmias and SCD.²¹⁰ It is worth noting that during heart failure, mitochondrial Ca^{2+} uptake/accumulation is actually reduced (because accumulated cytosolic Na^+ during heart failure inhibits mitochondrial Ca^{2+} uptake via activation of mitoNCX),^{119, 120} which also favors ROS production (by impairing mitochondrial energetics and oxidizing NAD(P)H).^{119, 120}

$\text{NO}^\bullet$ and peroxynitrite also play important roles in modulating cardiac Ca^{2+} channel and Ca^{2+} handling proteins. Increased $\text{NO}^\bullet$ inhibits L-type Ca^{2+} channel activity through increased S-nitrosylation^{211, 212} and cGMP-PKG-dependent phosphorylation²¹³ of Ca^{2+} channel subunits, which may contribute to the cardioprotective effects of IPC by limiting Ca^{2+} influx during ischemia.²¹² The effects of $\text{NO}^\bullet$ on RyR2 is concentration-dependent. Physiological $\text{NO}^\bullet$ levels ($\sim 1 \mu\text{M}$) do not affect RyR2 activity, whereas supraphysiological levels ($> 100 \mu\text{M}$) inhibit RyR2 activity.²¹⁴ Peroxynitrite, on the other hand, activates RyR2

by oxidizing its cysteine residues.²¹⁴ SERCA2a, by contrast, is inhibited by peroxynitrite-mediated tyrosine nitration. PKA pretreatment can prevent the inhibitory effects of peroxynitrite on SERCA2a by inducing dissociation of PLN from SERCA2a.²¹⁵

C. Myocardial ROS and potassium channels

Increased oxidative stress is known to inhibit repolarizing Kv currents including I_{to} and several delayed rectifier I_K (I_{Kr} , I_{Ks} and I_{Kur}) in mammalian ventricular myocytes.^{216–218} These effects are reversible by raising cellular antioxidant GSH levels.^{140, 219} ROS have been shown to regulate Kv current expression by reducing the transcript/protein expression of the Kv channel subunits²¹⁸ and by modulating the phosphorylation of these channel subunits through protein kinases such as PKA, PKC or protein tyrosine phosphatases.^{220–222} Cardiac Kv channels are also regulated by NO^* . It has been reported that NO^* inhibits human cardiac Kv4.3 channel, thereby reducing the transient outward K^+ current I_{to} .²²³ NO^* also blocks Kv1.5 channel through S-nitrosylation and the activation of cGMP/PKG pathway.²²⁴

Myocardial ROS also regulates myocyte membrane excitability through $sarK_{ATP}$ channels. During myocardial ischemia, fuel substrate deprivation and increased oxidative stress depolarize the mitochondrial network, leading to fluctuated Ψ_m and ATP production levels, which result in oscillation of $sarK_{ATP}$ currents and APDs.^{69, 225} The Ψ_m depolarization of the mitochondrial network is potentiated by widespread ROS production induced by focal increases in mitochondrial ROS (ROS-induced ROS release).^{60, 62, 226} IMAC plays a critical role in Ψ_m depolarization upon increased ROS; pharmacologic inhibition of IMAC has been shown to prevent Ψ_m depolarization and the oscillation of $sarK_{ATP}$ currents and APD,⁵⁹ as well as preventing ventricular arrhythmias in mammalian hearts^{227, 228} during myocardial ischemia.

Another group of K_{ATP} channels, residing on the mitochondrial inner membrane (mito K_{ATP} channels), contribute importantly to the protective effects of IPC, an endogenous cellular protective mechanism involving brief periods of ischemia that confers protection against infarction produced by a subsequent prolonged ischemia.^{148, 229} Under physiological conditions, mito K_{ATP} channel opening enhances mitochondrial ROS production, triggering downstream signaling pathways involved in gene transcription and cell growth.²³⁰ During IPC, the activation of mito K_{ATP} channels allows mitochondrial K^+ influx, resulting in partially depolarized Ψ_m , which leads to a compensatory increase in proton pumping and cellular respiration to maintain Ψ_m and ETC activity.²²⁹ Partially dissipated Ψ_m induced by mito K_{ATP} channel opening blunts mitochondrial Ca^{2+} accumulation during ischemia and reduces PTP opening upon reperfusion,^{231, 232} both of which contribute to the protection against cell death by IPC. The opening of mito K_{ATP} channel also triggers the production of “protective ROS”, which activates a PKC-dependent pathway that confers cardiac protection by reducing deleterious post-ischemic ROS production.^{233, 234} Interestingly, NO^* , a physiologically important free radical, has been shown to potentiate mito K_{ATP} opening²³⁵ and triggers both early and delayed IPC.^{236, 237} In addition, mito K_{ATP} opening during ischemia provides additional K^+ influx to maintain mitochondrial volume, which is critical to maintain the normal function of mitochondria and ETC.²³⁰ Pharmacologic inhibition of

mitoK_{ATP} channels abrogates the anti-arrhythmic effects of IPC²³⁸ and several mitoK_{ATP} channel openers are shown to provide protective effects against ischemia-induced cardiac arrhythmias.^{157, 239, 240}

D. Mitochondrial ROS and cardiac gap junction regulation

Cardiac gap junctions are formed by the assembly of a pair of juxtaposed intercellular hemichannels with each hemichannel consisting of six connexin (Cx) proteins. Gap junctions mediate the intercellular communication of small metabolites and ions and play an essential role in cardiac electrical conduction.²⁴¹ Among the three principal connexin isoforms (Cx40, Cx43 and Cx45) expressed in the heart, Cx43 is the predominant isoform expressed in ventricular myocytes.²⁴² Ventricular Cx43 expression has been shown to be downregulated with myocardial ischemia,^{243, 244} and heart failure,^{245, 246} which can lead to slowed conduction, increased electrical heterogeneity and abnormal anisotropic properties of the ventricles.^{247, 248} These changes can facilitate the initiation and maintenance of ventricular arrhythmias and SCDs.^{249, 250}

Cardiac renin-angiotensin system (RAS) activity is increased upon acute ischemia, contributing to the acute and chronic myocardial remodeling in response to ischemia.^{251, 252} RAS activation is known to increase myocardial oxidative stress and downregulate ventricular gap junction protein Cx43.^{253–255} Transgenic mouse models with enhanced cardiac RAS activity^{255, 256} have high incidence of conduction abnormality, ventricular arrhythmias and sudden death owing to reduced cardiac Cx43 and abnormal gap junction function.^{255, 256} Using a transgenic mouse model of cardiac-restricted angiotensin converting enzyme overexpression (ACE8/8),²⁵⁵ we have demonstrated that increased cardiac RAS activity leads to increased activity of cSrc, a redox-sensitive tyrosine kinase, by increasing cSrc phosphorylation at Tyr⁴¹⁶, in the ventricular myocardium. The activation of cSrc leads to Cx43 downregulation, impaired gap junction function, slowed cardiac conduction and increased incidence of ventricular arrhythmias and SCD.^{35, 257} The downregulation of Cx43 and increased risk for arrhythmias in ACE8/8 mice are alleviated by pharmacologic inhibition of RAS²⁵⁸ and cSrc.²⁵⁷ It has been shown that increased myocardial p-cSrc leads to Cx43 reduction through the competition between p-cSrc and Cx43 for the binding with zonula occludens-1, a scaffolding protein at the intercalated disk, resulting in Cx43 destabilization and degradation.²⁵⁹ Elevated p-cSrc levels also impair gap junction function by phosphorylating Cx43 at tyrosine residues.²⁶⁰ Using the same ACE8/8 mouse model, we have also shown that cardiac mitochondrial ROS were markedly increased with enhanced RAS activity.^{35, 257} Treatment with mitochondria-targeted antioxidant MitoTEMPO, but not with other types of antioxidants, decreases cSrc phosphorylation, preserves Cx43 expression, improves gap junction function, and abolishes ventricular arrhythmias and sudden cardiac death in ACE8/8 mice.³⁵ Although the clinical evidence suggests that exogenous antioxidants do not prevent SCD,²⁶¹ these data support a role for ROS, and suppression of endogenous, mitochondria-specific ROS may be an effective therapeutic approach.

Mechanistically, we have recently demonstrated that enhanced RAS signaling increases S-nitrosylation (SNO) of cardiac caveolin-1 (Cav1), an intrinsic inhibitor of cSrc, resulting in

Cav1-cSrc dissociation and subsequent cSrc activation. Cav1 SNO upon enhanced RAS signaling is mediated by increased Cav1-eNOS binding that is dependent on elevated mitochondrial ROS.²⁶² Consistent with these findings, Cav1 knock-out mice exhibit increased cSrc activation, reduced Cx43 expression, myocardial conduction defect and increased inducibility of ventricular arrhythmias.²⁶² Taken together, these data suggest the critical roles of mitochondrial oxidative stress and Cav1 in AngII-induced gap junction remodeling and arrhythmia. As mitochondrial ROS are increased in myocardial ischemia^{251, 252} and heart failure,^{56, 57} both of which are associated with RAS activation, reduced ventricular Cx43 and increased risk for ventricular arrhythmias and SCD, it would be of great interest to test if the treatment with mitochondria-targeted antioxidant can normalize Cx43 expression and prevent ventricular arrhythmias and SCD in these pathological conditions.

Effects of chronic versus acute metabolic derangement and oxidative stress on arrhythmogenicity

In this review, we have primarily focused on the impact of acute myocardial metabolic and oxidative stress on cardiac electrophysiology, arrhythmogenicity and SCDs. It is important to note that chronic conditions associated with metabolic derangements and excessive oxidative stress such as aging,²⁶³ hypoxia/obstructive sleep apnea,²⁶⁴ chronic kidney disease²⁶⁵ and diabetes,^{138, 139} also result in arrhythmogenic changes that predispose to SCDs. Many of the arrhythmogenic changes related to these chronic conditions involve electrical remodeling^{74, 75} and the creation of arrhythmogenic substrates such as fibrosis,^{266, 267} which may not be seen with acute metabolic and oxidative stress. Nevertheless, the effects of acute and chronic metabolic/oxidative stress on cardiac electrical function may not be mutually exclusive. The acute application of mitochondria-targeted antioxidant, for example, can restore the reduced peak I_{Na} observed with chronic heart failure.⁵⁷ In addition, reduced Kv currents in diabetic heart can be reversed by acute insulin treatment.¹⁴⁰ These observations suggest the contribution of the acute, modifiable metabolic/oxidative stress in these chronic conditions, which can be rapidly reversed by targeting the underlying metabolic/oxidative derangement. It is difficult to distinguish whether acute metabolic/oxidative changes exacerbate and amplify chronic changes or whether acute changes are unrelated to the chronic changes and simply occur on an arrhythmogenic background. Further studies are required to address these possibilities.

Conclusion

In summary, metabolic derangement and increased oxidative stress are prevalent in arrhythmogenic cardiac conditions, particularly during myocardial ischemia. Impaired cardiac metabolism and increased ROS production can lead to malfunction of various cellular mechanisms that are required to maintain normal electrical functioning and intracellular ionic homeostasis in cardiac myocytes. The impact of altered cardiac metabolism and increased oxidative stress on cardiac arrhythmogenicity is summarized in Table 1/Figure 4 and Table 2/Figure 5, respectively. As the conventional antiarrhythmic drugs targeting ion channels are often pro-arrhythmic, understanding the mechanisms

linking abnormal metabolism and oxidative stress to cardiac arrhythmias may help to develop novel therapeutics to reduce the risk of life-threatening arrhythmias and SCD in patients with cardiac diseases. These observations suggest that therapeutics tailored to ameliorate metabolic derangement and oxidative stress may prove a more efficacious alternative to traditional ion channel blocking drugs to address arrhythmia in associated with cardiac diseases and a list of potential novel therapeutics are summarized in Table 3.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Nonstandard Abbreviations and Acronyms

AMPK	adenosine monophosphate-activated protein kinase
APD	action potential duration
BH4	tetrahydrobiopterin
CaMKII	calcium/calmodulin-dependent protein kinase II
Cav1	caveolin-1
CHD	coronary heart disease
CoQ	coenzyme Q10
CoQH2	reduced coenzyme Q10
Cx	connexin
DAD	delayed afterdepolarization
DCM	dilated cardiomyopathy
Ψ_m	mitochondrial membrane potential
EAD	early afterdepolarization
ETC	electron-transport chain
FADH₂	flavin adenine dinucleotide
GSH	glutathione
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GPD1-L	glycerol-3-phosphate dehydrogenase 1-like
GSS	S-glutathiolation

H₂O₂	hydrogen peroxide
HOCl	hypochlorite
I_{CaL}	L-type Ca ²⁺ currents
I_K	delayed rectifier K ⁺ currents
I_{KATP}	ATP-sensitive K ⁺ current
IMAC	inner membrane anion channel
I_{Na}	Na ⁺ -current
IPC	ischemic preconditioning
I_{to}	transient outward K ⁺ current
K_{ir}	inwardly rectifying K ⁺ channels
K_v	voltage-gated K ⁺ channels
LPC	lysophosphatidylcholine
MnSOD	manganese superoxide dismutase
MCU	mitochondrial Ca ²⁺ uniporter
mitoNCX	mitochondrial Na ⁺ -Ca ²⁺ exchanger
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NCX	Na ⁺ /Ca ²⁺ exchanger
NHE	Na ⁺ /H ⁺ exchanger
NO[•]	nitric oxide
NOS	nitric oxide synthase
NOX2	NADPH oxidase 2
OXPHOS	oxidative phosphorylation
O₂^{•-}	superoxide
[•]OH	hydroxyl radicals
ONOO⁻	peroxynitrite
PEA	pulseless electrical activity
PI3Kα	phosphoinositide 3-kinase α
PKA	protein kinase A
PKC	protein kinase C
PKG	protein kinase G
PPARα	peroxisome proliferator-activated receptor α

PTP	mitochondrial permeability transition pore
RAS	renin-angiotensin system
RNS	reactive nitrogen species
ROS	reactive oxygen species
RyR2	ryanodine receptor 2
sarcK_{ATP}	sarcolemmal K _{ATP}
SCD	sudden cardiac death
SERCA	sarco/endoplasmic reticulum Ca ²⁺ -ATPase
SNO	S-nitrosylation
SR	sarcoplasmic reticulum
TCA cycle	tricarboxylic acid cycle
VF	ventricular fibrillation
VT	ventricular tachycardia

References

1. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Soliman EZ, Sorlie PD, Sotoodehnia N, Turan TN, Virani SS, Wong ND, Woo D, Turner MB. Heart disease and stroke statistics--2012 update: a report from the American Heart Association. *Circulation*. 2012; 125(1):e2–e220. [PubMed: 22179539]
2. Zheng ZJ, Croft JB, Giles WH, Mensah GA. Sudden cardiac death in the United States, 1989 to 1998. *Circulation*. 2001; 104(18):2158–2163. [PubMed: 11684624]
3. Zipes DP, Wellens HJ. Sudden cardiac death. *Circulation*. 1998; 98(21):2334–2351. [PubMed: 9826323]
4. Deo R, Albert CM. Epidemiology and genetics of sudden cardiac death. *Circulation*. 2012; 125(4):620–637. [PubMed: 22294707]
5. Bayes de Luna A, Coumel P, Leclercq JF. Ambulatory sudden cardiac death: mechanisms of production of fatal arrhythmia on the basis of data from 157 cases. *Am Heart J*. 1989; 117(1):151–159. [PubMed: 2911968]
6. Mitchell LB, Pineda EA, Titus JL, Bartosch PM, Benditt DG. Sudden death in patients with implantable cardioverter defibrillators: the importance of post-shock electromechanical dissociation. *J Am Coll Cardiol*. 2002; 39(8):1323–1328. [PubMed: 11955850]
7. Wood MA, Stambler BS, Damiano RJ, Greenway P, Ellenbogen KA. Lessons learned from data logging in a multicenter clinical trial using a late-generation implantable cardioverter-defibrillator. The Guardian ATP 4210 Multicenter Investigators Group. *J Am Coll Cardiol*. 1994; 24(7):1692–1699. [PubMed: 7963117]
8. Nichol G, Thomas E, Callaway CW, Hedges J, Powell JL, Aufderheide TP, Rea T, Lowe R, Brown T, Dreyer J, Davis D, Idris A, Stiell I. Regional variation in out-of-hospital cardiac arrest incidence and outcome. *JAMA*. 2008; 300(12):1423–1431. [PubMed: 18812533]
9. Teodorescu C, Reinier K, Dervan C, Uy-Evanado A, Samara M, Mariani R, Gunson K, Jui J, Chugh SS. Factors associated with pulseless electric activity versus ventricular fibrillation: the Oregon sudden unexpected death study. *Circulation*. 2010; 122(21):2116–2122. [PubMed: 21060069]

10. Bornfeldt KE, Tabas I. Insulin resistance, hyperglycemia, and atherosclerosis. *Cell Metab.* 2011; 14(5):575–585. [PubMed: 22055501]
11. Pansuria M, Xi H, Li L, Yang XF, Wang H. Insulin resistance, metabolic stress, and atherosclerosis. *Front Biosci (Schol Ed).* 2012; 4:916–931. [PubMed: 22202099]
12. Stocker R, Keaney JF Jr. Role of oxidative modifications in atherosclerosis. *Physiol Rev.* 2004; 84(4):1381–1478. [PubMed: 15383655]
13. Madamanchi NR, Vendrov A, Runge MS. Oxidative stress and vascular disease. *Arterioscler Thromb Vasc Biol.* 2005; 25(1):29–38. [PubMed: 15539615]
14. Hirooka Y, Sagara Y, Kishi T, Sunagawa K. Oxidative stress and central cardiovascular regulation. - Pathogenesis of hypertension and therapeutic aspects. *Circ J.* 2010; 74(5):827–835. [PubMed: 20424336]
15. Gerritsen J, Dekker JM, TenVoorde BJ, Kostense PJ, Heine RJ, Bouter LM, Heethaar RM, Stehouwer CD. Impaired autonomic function is associated with increased mortality, especially in subjects with diabetes, hypertension, or a history of cardiovascular disease: the Hoorn Study. *Diabetes Care.* 2001; 24(10):1793–1798. [PubMed: 11574444]
16. Priori SG, Aliot E, Blomstrom-Lundqvist C, Bossaert L, Breithardt G, Brugada P, Camm JA, Cappato R, Cobbe SM, Di Mario C, Maron BJ, McKenna WJ, Pedersen AK, Ravens U, Schwartz PJ, Trusz-Gluza M, Vardas P, Wellens HJ, Zipes DP. Update of the guidelines on sudden cardiac death of the European Society of Cardiology. *Eur Heart J.* 2003; 24(1):13–15. [PubMed: 12559931]
17. Dhalla NS, Adameova A, Kaur M. Role of catecholamine oxidation in sudden cardiac death. *Fundam Clin Pharmacol.* 2010; 24(5):539–546. [PubMed: 20584205]
18. Fozzard HA. Excitation-contraction coupling in the heart. *Adv Exp Med Biol.* 1991; 308:135–142. [PubMed: 1666263]
19. Roden DM, Balser JR, George AL Jr, Anderson ME. Cardiac ion channels. *Annu Rev Physiol.* 2002; 64:431–475. [PubMed: 11826275]
20. Nerbonne JM, Kass RS. Molecular physiology of cardiac repolarization. *Physiol Rev.* 2005; 85(4):1205–1253. [PubMed: 16183911]
21. Kanno S, Saffitz JE. The role of myocardial gap junctions in electrical conduction and arrhythmogenesis. *Cardiovasc Pathol.* 2001; 10(4):169–177. [PubMed: 11600334]
22. Harris DA, Das AM. Control of mitochondrial ATP synthesis in the heart. *Biochem J.* 1991; 280 (Pt 3):561–573. [PubMed: 1837214]
23. Schaper J, Meiser E, Stammeler G. Ultrastructural morphometric analysis of myocardium from dogs, rats, hamsters, mice, and from human hearts. *Circ Res.* 1985; 56(3):377–391. [PubMed: 3882260]
24. Chen LB. Mitochondrial membrane potential in living cells. *Annu Rev Cell Biol.* 1988; 4:155–181. [PubMed: 3058159]
25. Gertz EW, Wisneski JA, Stanley WC, Neese RA. Myocardial substrate utilization during exercise in humans. Dual carbon-labeled carbohydrate isotope experiments. *J Clin Invest.* 1988; 82(6):2017–2025. [PubMed: 3198763]
26. Wisneski JA, Gertz EW, Neese RA, Gruenke LD, Morris DL, Craig JC. Metabolic fate of extracted glucose in normal human myocardium. *J Clin Invest.* 1985; 76(5):1819–1827. [PubMed: 4056055]
27. Schramm M, Klieber HG, Daut J. The energy expenditure of actomyosin-ATPase, Ca^{2+} -ATPase and Na^{+} , K^{+} -ATPase in guinea-pig cardiac ventricular muscle. *J Physiol.* 1994; 481 (Pt 3):647–662. [PubMed: 7707233]
28. Overend CL, Eisner DA, O'Neill SC. Altered cardiac sarcoplasmic reticulum function of intact myocytes of rat ventricle during metabolic inhibition. *Circ Res.* 2001; 88(2):181–187. [PubMed: 11157670]
29. Silverman HS, Stern MD. Ionic basis of ischaemic cardiac injury: insights from cellular studies. *Cardiovasc Res.* 1994; 28(5):581–597. [PubMed: 8025901]
30. Pung YF, Sam WJ, Stevanov K, Enrick M, Chen CL, Kolz C, Thakker P, Hardwick JP, Chen YR, Dyck JR, Yin L, Chilian WM. Mitochondrial oxidative stress corrupts coronary collateral growth

by activating adenosine monophosphate activated kinase- α signaling. *Arterioscler Thromb Vasc Biol.* 2013; 33(8):1911–1919. [PubMed: 23788766]

31. Sripathi SR, He W, Atkinson CL, Smith JJ, Liu Z, Elledge BM, Jahng WJ. Mitochondrial-nuclear communication by prohibitin shuttling under oxidative stress. *Biochemistry.* 2011; 50(39):8342–8351. [PubMed: 21879722]
32. Sato T, Sasaki N, Seharaseyon J, O'Rourke B, Marban E. Selective pharmacological agents implicate mitochondrial but not sarcolemmal K_{ATP} channels in ischemic cardioprotection. *Circulation.* 2000; 101(20):2418–2423. [PubMed: 10821820]
33. Prosser BL, Ward CW, Lederer WJ. X-ROS signaling: rapid mechano-chemo transduction in heart. *Science.* 2011; 333(6048):1440–1445. [PubMed: 21903813]
34. Jian Z, Han H, Zhang T, Puglisi J, Izu LT, Shaw JA, Onofriok E, Erickson JR, Chen YJ, Horvath B, Shinkunas R, Xiao W, Li Y, Pan T, Chan J, Banyasz T, Tardiff JC, Chiamvimonvat N, Bers DM, Lam KS, Chen-Izu Y. Mechanochemotransduction during cardiac myocytes contraction is mediated by localized nitric oxide signaling. *Sci Signal.* 2014; 7(317):ra27. [PubMed: 24643800]
35. Sovari AA, Rutledge CA, Jeong EM, Dolmatova E, Arasu D, Liu H, Vahdani N, Gu L, Zandieh S, Xiao L, Bonini MG, Duffy HS, Dudley SC Jr. Mitochondria oxidative stress, connexin43 remodeling, and sudden arrhythmic death. *Circ Arrhythm Electrophysiol.* 2013; 6(3):623–631. [PubMed: 23559673]
36. Sanders PN, Koval OM, Jaffer OA, Prasad AM, Businga TR, Scott JA, Hayden PJ, Luczak ED, Dickey DD, Allamargot C, Olivier AK, Meyerholz DK, Robison AJ, Winder DG, Blackwell TS, Dworski R, Sammut D, Wagner BA, Buettner GR, Pope RM, Miller FJ Jr, Dibbern ME, Haitchi HM, Mohler PJ, Howarth PH, Zabner J, Kline JN, Grumbach IM, Anderson ME. CaMKII is essential for the proasthmatic effects of oxidation. *Science translational medicine.* 2013; 5(195):195ra197.
37. Zafari AM, Harrison DG. Free radicals in heart failure: therapeutic targets for old and new drugs. *Congest Heart Fail.* 2002; 8(3):129–130. [PubMed: 12045380]
38. Aggarwal NT, Makielski JC. Redox control of cardiac excitability. *Antioxid Redox Signal.* 2013; 18(4):432–468. [PubMed: 22897788]
39. Yang KC, Bonini MG, Dudley SC Jr. Mitochondria and arrhythmias. *Free Radic Biol Med.* 2014; 71:351–361. [PubMed: 24713422]
40. Griendling KK, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. *Circ Res.* 2000; 86(5):494–501. [PubMed: 10720409]
41. Harrison R. Structure and function of xanthine oxidoreductase: where are we now? *Free Radic Biol Med.* 2002; 33(6):774–797. [PubMed: 12208366]
42. Landmesser U, Dikalov S, Price SR, McCann L, Fukui T, Holland SM, Mitch WE, Harrison DG. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J Clin Invest.* 2003; 111(8):1201–1209. [PubMed: 12697739]
43. Chen CA, Wang TY, Varadharaj S, Reyes LA, Hemann C, Talukder MA, Chen YR, Druhan LJ, Zweier JL. S-glutathionylation uncouples eNOS and regulates its cellular and vascular function. *Nature.* 2010; 468(7327):1115–1118. [PubMed: 21179168]
44. Klatt P, Lamas S. Regulation of protein function by S-glutathiolation in response to oxidative and nitrosative stress. *Eur J Biochem.* 2000; 267(16):4928–4944. [PubMed: 10931175]
45. Stamler JS, Lamas S, Fang FC. Nitrosylation. the prototypic redox-based signaling mechanism. *Cell.* 2001; 106(6):675–683. [PubMed: 11572774]
46. Turrens JF. Mitochondrial formation of reactive oxygen species. *J Physiol.* 2003; 552(Pt 2):335–344. [PubMed: 14561818]
47. Murphy MP. How mitochondria produce reactive oxygen species. *Biochem J.* 2009; 417(1):1–13. [PubMed: 19061483]
48. Boveris A, Chance B. The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. *Biochem J.* 1973; 134(3):707–716. [PubMed: 4749271]
49. Schofield CJ, Ratcliffe PJ. Oxygen sensing by HIF hydroxylases. *Nat Rev Mol Cell Biol.* 2004; 5(5):343–354. [PubMed: 15122348]
50. Weisiger RA, Fridovich I. Superoxide dismutase. Organelle specificity. *J Biol Chem.* 1973; 248(10):3582–3592. [PubMed: 4702877]

51. Aon MA, Cortassa S, O'Rourke B. Redox-optimized ROS balance: a unifying hypothesis. *Biochim Biophys Acta*. 2010; 1797(6–7):865–877. [PubMed: 20175987]
52. Cortassa S, O'Rourke B, Aon MA. Redox-optimized ROS balance and the relationship between mitochondrial respiration and ROS. *Biochim Biophys Acta*. 2014; 1837(2):287–295. [PubMed: 24269780]
53. Nickel A, Kohlhaas M, Maack C. Mitochondrial reactive oxygen species production and elimination. *J Mol Cell Cardiol*. 2014; 73:26–33. [PubMed: 24657720]
54. Novalija E, Kevin LG, Eells JT, Henry MM, Stowe DF. Anesthetic preconditioning improves adenosine triphosphate synthesis and reduces reactive oxygen species formation in mitochondria after ischemia by a redox dependent mechanism. *Anesthesiology*. 2003; 98(5):1155–1163. [PubMed: 12717137]
55. Tompkins AJ, Burwell LS, Digerness SB, Zaragoza C, Holman WL, Brookes PS. Mitochondrial dysfunction in cardiac ischemia-reperfusion injury: ROS from complex I, without inhibition. *Biochim Biophys Acta*. 2006; 1762(2):223–231. [PubMed: 16278076]
56. Ide T, Tsutsui H, Kinugawa S, Utsumi H, Kang D, Hattori N, Uchida K, Arimura K, Egashira K, Takeshita A. Mitochondrial electron transport complex I is a potential source of oxygen free radicals in the failing myocardium. *Circ Res*. 1999; 85(4):357–363. [PubMed: 10455064]
57. Liu M, Gu L, Sulkin MS, Liu H, Jeong EM, Greener I, Xie A, Efimov IR, Dudley SC Jr. Mitochondrial dysfunction causing cardiac sodium channel downregulation in cardiomyopathy. *J Mol Cell Cardiol*. 2013; 54:25–34. [PubMed: 23123323]
58. Brenner C, Moulin M. Physiological roles of the permeability transition pore. *Circ Res*. 2012; 111(9):1237–1247. [PubMed: 23065346]
59. Aon MA, Cortassa S, Marban E, O'Rourke B. Synchronized whole cell oscillations in mitochondrial metabolism triggered by a local release of reactive oxygen species in cardiac myocytes. *J Biol Chem*. 2003; 278(45):44735–44744. [PubMed: 12930841]
60. Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial ROS-induced ROS release: an update and review. *Biochim Biophys Acta*. 2006; 1757(5–6):509–517. [PubMed: 16829228]
61. Brady NR, Hamacher-Brady A, Westerhoff HV, Gottlieb RA. A wave of reactive oxygen species (ROS)-induced ROS release in a sea of excitable mitochondria. *Antioxid Redox Signal*. 2006; 8(9–10):1651–1665. [PubMed: 16987019]
62. Akar FG, Aon MA, Tomaselli GF, O'Rourke B. The mitochondrial origin of postischemic arrhythmias. *J Clin Invest*. 2005; 115(12):3527–3535. [PubMed: 16284648]
63. Halestrap AP, Richardson AP. The mitochondrial permeability transition: A current perspective on its identity and role in ischaemia/reperfusion injury. *J Mol Cell Cardiol*. 2014
64. Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol Rev*. 2014; 94(3):909–950. [PubMed: 24987008]
65. Argaud L, Gateau-Roesch O, Muntean D, Chalabreysse L, Loufouat J, Robert D, Ovize M. Specific inhibition of the mitochondrial permeability transition prevents lethal reperfusion injury. *J Mol Cell Cardiol*. 2005; 38(2):367–374. [PubMed: 15698843]
66. Gomez L, Thibault H, Gharib A, Dumont JM, Vuagniaux G, Scalfaro P, Derumeaux G, Ovize M. Inhibition of mitochondrial permeability transition improves functional recovery and reduces mortality following acute myocardial infarction in mice. *Am J Physiol Heart Circ Physiol*. 2007; 293(3):H1654–1661. [PubMed: 17557911]
67. Giorgio V, von Stockum S, Antoniel M, Fabbro A, Fogolari F, Forte M, Glick GD, Petronilli V, Zoratti M, Szabo I, Lippe G, Bernardi P. Dimers of mitochondrial ATP synthase form the permeability transition pore. *Proc Natl Acad Sci U S A*. 2013; 110(15):5887–5892. [PubMed: 23530243]
68. Bonora M, Bononi A, De Marchi E, Giorgi C, Lebedzinska M, Marchi S, Patergnani S, Rimessi A, Suski JM, Wojtala A, Wieckowski MR, Kroemer G, Galluzzi L, Pinton P. Role of the c subunit of the FO ATP synthase in mitochondrial permeability transition. *Cell Cycle*. 2013; 12(4):674–683. [PubMed: 23343770]
69. Aon MA, Cortassa S, O'Rourke B. Mitochondrial oscillations in physiology and pathophysiology. *Adv Exp Med Biol*. 2008; 641:98–117. [PubMed: 18783175]

70. Murray J, Taylor SW, Zhang B, Ghosh SS, Capaldi RA. Oxidative damage to mitochondrial complex I due to peroxynitrite: identification of reactive tyrosines by mass spectrometry. *J Biol Chem.* 2003; 278(39):37223–37230. [PubMed: 12857734]
71. Tatarkova Z, Kuka S, Racay P, Lehotsky J, Dobrota D, Mistuna D, Kaplan P. Effects of aging on activities of mitochondrial electron transport chain complexes and oxidative damage in rat heart. *Physiol Res.* 2011; 60(2):281–289. [PubMed: 21114360]
72. Echtay KS, Roussel D, St-Pierre J, Jekabsons MB, Cadenas S, Stuart JA, Harper JA, Roebuck SJ, Morrison A, Pickering S, Clapham JC, Brand MD. Superoxide activates mitochondrial uncoupling proteins. *Nature.* 2002; 415(6867):96–99. [PubMed: 11780125]
73. Echtay KS, Esteves TC, Pakay JL, Jekabsons MB, Lambert AJ, Portero-Otin M, Pamplona R, Vidal-Puig AJ, Wang S, Roebuck SJ, Brand MD. A signalling role for 4-hydroxy-2-nonenal in regulation of mitochondrial uncoupling. *EMBO J.* 2003; 22(16):4103–4110. [PubMed: 12912909]
74. Aiba T, Tomaselli GF. Electrical remodeling in the failing heart. *Curr Opin Cardiol.* 2010; 25(1): 29–36. [PubMed: 19907317]
75. Cutler MJ, Jeyaraj D, Rosenbaum DS. Cardiac electrical remodeling in health and disease. *Trends Pharmacol Sci.* 2011; 32(3):174–180. [PubMed: 21316769]
76. Bers DM. Calcium cycling and signaling in cardiac myocytes. *Annu Rev Physiol.* 2008; 70:23–49. [PubMed: 17988210]
77. Varadarajan SG, An J, Novalija E, Smart SC, Stowe DF. Changes in $[Na^+]_i$, compartmental $[Ca^{2+}]$, and NADH with dysfunction after global ischemia in intact hearts. *Am J Physiol Heart Circ Physiol.* 2001; 280(1):H280–293. [PubMed: 11123243]
78. An J, Varadarajan SG, Novalija E, Stowe DF. Ischemic and anesthetic preconditioning reduces cytosolic $[Ca^{2+}]$ and improves Ca^{2+} responses in intact hearts. *Am J Physiol Heart Circ Physiol.* 2001; 281(4):H1508–1523. [PubMed: 11557539]
79. Moore RL, Yelamarty RV, Misawa H, Scaduto RC Jr, Pawlusch DG, Elensky M, Cheung JY. Altered Ca^{2+} dynamics in single cardiac myocytes from renovascular hypertensive rats. *Am J Physiol.* 1991; 260(2 Pt 1):C327–337. [PubMed: 1825451]
80. Bentivegna LA, Ablin LW, Kihara Y, Morgan JP. Altered calcium handling in left ventricular pressure-overload hypertrophy as detected with aequorin in the isolated, perfused ferret heart. *Circ Res.* 1991; 69(6):1538–1545. [PubMed: 1835431]
81. O'Rourke B, Kass DA, Tomaselli GF, Kaab S, Tunin R, Marban E. Mechanisms of altered excitation-contraction coupling in canine tachycardia-induced heart failure, I: experimental studies. *Circ Res.* 1999; 84(5):562–570. [PubMed: 10082478]
82. Pogwizd SM, Qi M, Yuan W, Samarel AM, Bers DM. Upregulation of Na^+/Ca^{2+} exchanger expression and function in an arrhythmogenic rabbit model of heart failure. *Circ Res.* 1999; 85(11):1009–1019. [PubMed: 10571531]
83. Koretsune Y, Marban E. Mechanism of ischemic contracture in ferret hearts: relative roles of $[Ca^{2+}]_i$ elevation and ATP depletion. *Am J Physiol.* 1990; 258(1 Pt 2):H9–16. [PubMed: 2301617]
84. Elliott AC, Smith GL, Eisner DA, Allen DG. Metabolic changes during ischaemia and their role in contractile failure in isolated ferret hearts. *J Physiol.* 1992; 454:467–490. [PubMed: 1474498]
85. Murphy E, Steenbergen C, Levy LA, Raju B, London RE. Cytosolic free magnesium levels in ischemic rat heart. *J Biol Chem.* 1989; 264(10):5622–5627. [PubMed: 2925624]
86. Eisner DA, Richards DE. Inhibition of the sodium pump by inorganic phosphate in resealed red cell ghosts. *J Physiol.* 1982; 326:1–10. [PubMed: 6286945]
87. Apell HJ, Nelson MT, Marcus MM, Lauger P. Effects of the ATP, ADP and inorganic phosphate on the transport rate of the Na^+ , K^+ -pump. *Biochim Biophys Acta.* 1986; 857(1):105–115. [PubMed: 3008841]
88. Undrovinas AI, Fleidervish IA, Makielski JC. Inward sodium current at resting potentials in single cardiac myocytes induced by the ischemic metabolite lysophosphatidylcholine. *Circ Res.* 1992; 71(5):1231–1241. [PubMed: 1327577]
89. Jones DK, Peters CH, Tolhurst SA, Claydon TW, Ruben PC. Extracellular proton modulation of the cardiac voltage-gated sodium channel, $Nav_{1.5}$. *Biophys J.* 2011; 101(9):2147–2156. [PubMed: 22067152]

90. Sokolov S, Peters CH, Rajamani S, Ruben PC. Proton-dependent inhibition of the cardiac sodium channel Nav1.5 by ranolazine. *Front Pharmacol.* 2013; 4:78. [PubMed: 23801963]
91. Valdivia CR, Chu WW, Pu J, Foell JD, Haworth RA, Wolff MR, Kamp TJ, Makielski JC. Increased late sodium current in myocytes from a canine heart failure model and from failing human heart. *J Mol Cell Cardiol.* 2005; 38(3):475–483. [PubMed: 15733907]
92. Mochizuki S, MacLeod KT. Effects of hypoxia and metabolic inhibition on increases in intracellular Ca^{2+} concentration induced by $\text{Na}^+/\text{Ca}^{2+}$ exchange in isolated guinea-pig cardiac myocytes. *J Mol Cell Cardiol.* 1997; 29(11):2979–2987. [PubMed: 9405173]
93. Weber CR, Piacentino V 3rd, Houser SR, Bers DM. Dynamic regulation of sodium/calcium exchange function in human heart failure. *Circulation.* 2003; 108(18):2224–2229. [PubMed: 14557358]
94. Armoundas AA, Hobai IA, Tomaselli GF, Winslow RL, O'Rourke B. Role of sodium-calcium exchanger in modulating the action potential of ventricular myocytes from normal and failing hearts. *Circ Res.* 2003; 93(1):46–53. [PubMed: 12805237]
95. Xu L, Mann G, Meissner G. Regulation of cardiac Ca^{2+} release channel (ryanodine receptor) by Ca^{2+} , H^+ , Mg^{2+} , and adenine nucleotides under normal and simulated ischemic conditions. *Circ Res.* 1996; 79(6):1100–1109. [PubMed: 8943948]
96. Ferrier GR, Saunders JH, Mendez C. A cellular mechanism for the generation of ventricular arrhythmias by acetylcholinesterase. *Circ Res.* 1973; 32(5):600–609. [PubMed: 4713202]
97. Clusin WT. Mechanisms of calcium transient and action potential alternans in cardiac cells and tissues. *Am J Physiol Heart Circ Physiol.* 2008; 294(1):H1–H10. [PubMed: 17951365]
98. Qian YW, Clusin WT, Lin SF, Han J, Sung RJ. Spatial heterogeneity of calcium transient alternans during the early phase of myocardial ischemia in the blood-perfused rabbit heart. *Circulation.* 2001; 104(17):2082–2087. [PubMed: 11673350]
99. Palty R, Silverman WF, Hershfinkel M, Caporale T, Sensi SL, Parnis J, Nolte C, Fishman D, Shoshan-Barmatz V, Herrmann S, Khananshvili D, Sekler I. NCLX is an essential component of mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchange. *Proc Natl Acad Sci U S A.* 2010; 107(1):436–441. [PubMed: 20018762]
100. Maack C, Cortassa S, Aon MA, Ganesan AN, Liu T, O'Rourke B. Elevated cytosolic Na^+ decreases mitochondrial Ca^{2+} uptake during excitation-contraction coupling and impairs energetic adaptation in cardiac myocytes. *Circ Res.* 2006; 99(2):172–182. [PubMed: 16778127]
101. Drago I, De Stefani D, Rizzuto R, Pozzan T. Mitochondrial Ca^{2+} uptake contributes to buffering cytoplasmic Ca^{2+} peaks in cardiac myocytes. *Proc Natl Acad Sci U S A.* 2012; 109(32):12986–12991. [PubMed: 22822213]
102. Williams GS, Boyman L, Chikando AC, Khairallah RJ, Lederer WJ. Mitochondrial calcium uptake. *Proc Natl Acad Sci U S A.* 2013; 110(26):10479–10486. [PubMed: 23759742]
103. O'Rourke B, Blatter LA. Mitochondrial Ca^{2+} uptake: tortoise or hare? *J Mol Cell Cardiol.* 2009; 46(6):767–774. [PubMed: 19162034]
104. Florea SM, Blatter LA. The role of mitochondria for the regulation of cardiac alternans. *Front Physiol.* 2010; 1:141. [PubMed: 21423381]
105. de Garcia-Rivas GJ, Carvajal K, Correa F, Zazueta C. Ru360, a specific mitochondrial calcium uptake inhibitor, improves cardiac post-ischaemic functional recovery in rats in vivo. *Br J Pharmacol.* 2006; 149(7):829–837. [PubMed: 17031386]
106. Liu T, O'Rourke B. Enhancing mitochondrial Ca^{2+} uptake in myocytes from failing hearts restores energy supply and demand matching. *Circ Res.* 2008; 103(3):279–288. [PubMed: 18599868]
107. Haussinger D, Schliess F. Osmotic induction of signaling cascades: role in regulation of cell function. *Biochem Biophys Res Commun.* 1999; 255(3):551–555. [PubMed: 10049748]
108. Haigney MC, Lakatta EG, Stern MD, Silverman HS. Sodium channel blockade reduces hypoxic sodium loading and sodium-dependent calcium loading. *Circulation.* 1994; 90(1):391–399. [PubMed: 8026023]
109. Ingwall JS. How high does intracellular sodium rise during acute myocardial ischaemia? A view from NMR spectroscopy. *Cardiovasc Res.* 1995; 29(2):279. [PubMed: 7736509]

110. ten Hove M, van Emous JG, van Echteld CJ. Na^+ overload during ischemia and reperfusion in rat hearts: comparison of the Na^+/H^+ exchange blockers EIPA, cariporide and eniporide. *Mol Cell Biochem.* 2003; 250(1–2):47–54. [PubMed: 12962142]
111. Wang Y, Meyer JW, Ashraf M, Shull GE. Mice with a null mutation in the NHE1 Na^+/H^+ exchanger are resistant to cardiac ischemia-reperfusion injury. *Circ Res.* 2003; 93(8):776–782. [PubMed: 12970112]
112. John SA, Kondo R, Wang SY, Goldhaber JJ, Weiss JN. Connexin-43 hemichannels opened by metabolic inhibition. *J Biol Chem.* 1999; 274(1):236–240. [PubMed: 9867835]
113. Retamal MA, Schalper KA, Shoji KF, Bennett MV, Saez JC. Opening of connexin 43 hemichannels is increased by lowering intracellular redox potential. *Proc Natl Acad Sci U S A.* 2007; 104(20):8322–8327. [PubMed: 17494739]
114. Kondo RP, Wang SY, John SA, Weiss JN, Goldhaber JJ. Metabolic inhibition activates a non-selective current through connexin hemichannels in isolated ventricular myocytes. *J Mol Cell Cardiol.* 2000; 32(10):1859–1872. [PubMed: 11013130]
115. Manjunath CK, Page E. Cell biology and protein composition of cardiac gap junctions. *Am J Physiol.* 1985; 248(6 Pt 2):H783–791. [PubMed: 2408491]
116. Jung DW, Apel LM, Brierley GP. Transmembrane gradients of free Na^+ in isolated heart mitochondria estimated using a fluorescent probe. *Am J Physiol.* 1992; 262(4 Pt 1):C1047–1055. [PubMed: 1566810]
117. Kim B, Matsuoka S. Cytoplasmic Na^+ -dependent modulation of mitochondrial Ca^{2+} via electrogenic mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchange. *J Physiol.* 2008; 586(6):1683–1697. [PubMed: 18218682]
118. Donoso P, Mill JG, O'Neill SC, Eisner DA. Fluorescence measurements of cytoplasmic and mitochondrial sodium concentration in rat ventricular myocytes. *J Physiol.* 1992; 448:493–509. [PubMed: 1593474]
119. Liu T, Takimoto E, Dimaano VL, DeMazumder D, Kettlewell S, Smith G, Sidor A, Abraham TP, O'Rourke B. Inhibiting mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchange prevents sudden death in a Guinea pig model of heart failure. *Circ Res.* 2014; 115(1):44–54. [PubMed: 24780171]
120. Kohlhaas M, Liu T, Knopp A, Zeller T, Ong MF, Bohm M, O'Rourke B, Maack C. Elevated cytosolic Na^+ increases mitochondrial formation of reactive oxygen species in failing cardiac myocytes. *Circulation.* 2010; 121(14):1606–1613. [PubMed: 20351235]
121. Ward CA, Giles WR. Ionic mechanism of the effects of hydrogen peroxide in rat ventricular myocytes. *J Physiol.* 1997; 500 (Pt 3):631–642. [PubMed: 9161981]
122. Ma JH, Luo AT, Zhang PH. Effect of hydrogen peroxide on persistent sodium current in guinea pig ventricular myocytes. *Acta Pharmacol Sin.* 2005; 26(7):828–834. [PubMed: 15960889]
123. Liu M, Liu H, Dudley SC Jr. Reactive oxygen species originating from mitochondria regulate the cardiac sodium channel. *Circ Res.* 2010; 107(8):967–974. [PubMed: 20724705]
124. Wagner S, Ruff HM, Weber SL, Bellmann S, Sowa T, Schulte T, Anderson ME, Grandi E, Bers DM, Backs J, Belardinelli L, Maier LS. Reactive oxygen species-activated Ca/calmodulin kinase II δ is required for late $\text{I}(\text{Na})$ augmentation leading to cellular Na and Ca overload. *Circ Res.* 2011; 108(5):555–565. [PubMed: 21252154]
125. Bay J, Kohlhaas M, Maack C. Intracellular Na^+ and cardiac metabolism. *J Mol Cell Cardiol.* 2013; 61:20–27. [PubMed: 23727097]
126. Makielski JC, Farley AL. Na^+ current in human ventricle: implications for sodium loading and homeostasis. *J Cardiovasc Electrophysiol.* 2006; 17 (Suppl 1):S15–S20. [PubMed: 16686671]
127. Shander GS, Undrovinas AI, Makielski JC. Rapid onset of lysophosphatidylcholine-induced modification of whole cell cardiac sodium current kinetics. *J Mol Cell Cardiol.* 1996; 28(4):743–753. [PubMed: 8732502]
128. Hardie DG, Carling D. The AMP-activated protein kinase--fuel gauge of the mammalian cell? *Eur J Biochem.* 1997; 246(2):259–273. [PubMed: 9208914]
129. Hardie DG. AMP-activated protein kinase: the guardian of cardiac energy status. *J Clin Invest.* 2004; 114(4):465–468. [PubMed: 15314681]

130. Light PE, Wallace CH, Dyck JR. Constitutively active adenosine monophosphate-activated protein kinase regulates voltage-gated sodium channels in ventricular myocytes. *Circulation*. 2003; 107(15):1962–1965. [PubMed: 12682004]
131. Gollob MH, Green MS, Tang AS, Gollob T, Karibe A, Ali Hassan AS, Ahmad F, Lozado R, Shah G, Fananapazir L, Bachinski LL, Roberts R. Identification of a gene responsible for familial Wolff-Parkinson-White syndrome. *N Engl J Med*. 2001; 344(24):1823–1831. [PubMed: 11407343]
132. Gollob MH, Seger JJ, Gollob TN, Tapscott T, Gonzales O, Bachinski L, Roberts R. Novel PRKAG2 mutation responsible for the genetic syndrome of ventricular preexcitation and conduction system disease with childhood onset and absence of cardiac hypertrophy. *Circulation*. 2001; 104(25):3030–3033. [PubMed: 11748095]
133. Nichols CG, Lopatin AN. Inward rectifier potassium channels. *Annu Rev Physiol*. 1997; 59:171–191. [PubMed: 9074760]
134. Lopatin AN, Nichols CG. Inward rectifiers in the heart: an update on I_{K1} . *J Mol Cell Cardiol*. 2001; 33(4):625–638. [PubMed: 11273717]
135. Fan Z, Makielski JC. Intracellular H^+ and Ca^{2+} modulation of trypsin-modified ATP-sensitive K^+ channels in rabbit ventricular myocytes. *Circ Res*. 1993; 72(3):715–722. [PubMed: 8381726]
136. Zhuo ML, Huang Y, Liu DP, Liang CC. K_{ATP} channel: relation with cell metabolism and role in the cardiovascular system. *Int J Biochem Cell Biol*. 2005; 37(4):751–764. [PubMed: 15694835]
137. Suzuki M, Saito T, Sato T, Tamagawa M, Miki T, Seino S, Nakaya H. Cardioprotective effect of diazoxide is mediated by activation of sarcolemmal but not mitochondrial ATP-sensitive potassium channels in mice. *Circulation*. 2003; 107(5):682–685. [PubMed: 12578868]
138. Veglio M, Chinaglia A, Cavallo-Perin P. QT interval, cardiovascular risk factors and risk of death in diabetes. *J Endocrinol Invest*. 2004; 27(2):175–181. [PubMed: 15129815]
139. Aksnes TA, Schmieder RE, Kjeldsen SE, Ghani S, Hua TA, Julius S. Impact of new-onset diabetes mellitus on development of atrial fibrillation and heart failure in high-risk hypertension (from the VALUE Trial). *Am J Cardiol*. 2008; 101(5):634–638. [PubMed: 18308012]
140. Xu Z, Patel KP, Lou MF, Rozanski GJ. Up-regulation of K^+ channels in diabetic rat ventricular myocytes by insulin and glutathione. *Cardiovasc Res*. 2002; 53(1):80–88. [PubMed: 11744015]
141. Xu Z, Patel KP, Rozanski GJ. Metabolic basis of decreased transient outward K^+ current in ventricular myocytes from diabetic rats. *Am J Physiol*. 1996; 271(5 Pt 2):H2190–2196. [PubMed: 8945940]
142. Shimoni Y, Firek L, Severson D, Giles W. Short-term diabetes alters K^+ currents in rat ventricular myocytes. *Circ Res*. 1994; 74(4):620–628. [PubMed: 8137498]
143. Xu Z, Rozanski GJ. K^+ current inhibition by amphiphilic fatty acid metabolites in rat ventricular myocytes. *Am J Physiol*. 1998; 275(6 Pt 1):C1660–1667. [PubMed: 9843728]
144. Marionneau C, Aimond F, Brunet S, Niwa N, Finck B, Kelly DP, Nerbonne JM. PPAR α -mediated remodeling of repolarizing voltage-gated K^+ (K_v) channels in a mouse model of metabolic cardiomyopathy. *J Mol Cell Cardiol*. 2008; 44(6):1002–1015. [PubMed: 18482733]
145. Yang KC, Foeger NC, Marionneau C, Jay PY, McMullen JR, Nerbonne JM. Homeostatic regulation of electrical excitability in physiological cardiac hypertrophy. *J Physiol*. 2010; 588(Pt 24):5015–5032. [PubMed: 20974681]
146. Yang KC, Jay PY, McMullen JR, Nerbonne JM. Enhanced cardiac PI3K α signalling mitigates arrhythmogenic electrical remodelling in pathological hypertrophy and heart failure. *Cardiovasc Res*. 2012; 93(2):252–262. [PubMed: 22038742]
147. Yang KC, Tseng YT, Nerbonne JM. Exercise training and PI3K α -induced electrical remodeling is independent of cellular hypertrophy and Akt signaling. *J Mol Cell Cardiol*. 2012; 53(4):532–541. [PubMed: 22824041]
148. O'Rourke B. Myocardial K_{ATP} channels in preconditioning. *Circ Res*. 2000; 87(10):845–855. [PubMed: 11073879]
149. Wu J, Cui N, Piao H, Wang Y, Xu H, Mao J, Jiang C. Allosteric modulation of the mouse Kir6.2 channel by intracellular H^+ and ATP. *J Physiol*. 2002; 543(Pt 2):495–504. [PubMed: 12205184]

150. Gettes LS, Cascio WE, Johnson T, Fleet WF. Local myocardial biochemical and ionic alterations during myocardial ischaemia and reperfusion. *Drugs*. 1991; 42 (Suppl 1):7–13. [PubMed: 1718696]
151. Steenbergen C, Murphy E, Watts JA, London RE. Correlation between cytosolic free calcium, contracture, ATP, and irreversible ischemic injury in perfused rat heart. *Circ Res*. 1990; 66(1): 135–146. [PubMed: 2295135]
152. Faivre JF, Findlay I. Action potential duration and activation of ATP-sensitive potassium current in isolated guinea-pig ventricular myocytes. *Biochim Biophys Acta*. 1990; 1029(1):167–172. [PubMed: 2223807]
153. Bhatnagar A. Contribution of ATP to oxidative stress-induced changes in action potential of isolated cardiac myocytes. *Am J Physiol*. 1997; 272(4 Pt 2):H1598–1608. [PubMed: 9139941]
154. Aon MA, Cortassa S, Akar FG, Brown DA, Zhou L, O'Rourke B. From mitochondrial dynamics to arrhythmias. *Int J Biochem Cell Biol*. 2009; 41(10):1940–1948. [PubMed: 19703656]
155. Vajda S, Baczkó I, Lepran I. Selective cardiac plasma-membrane K_{ATP} channel inhibition is defibrillatory and improves survival during acute myocardial ischemia and reperfusion. *Eur J Pharmacol*. 2007; 577(1–3):115–123. [PubMed: 17904545]
156. Fischbach PS, White A, Barrett TD, Lucchesi BR. Risk of ventricular proarrhythmia with selective opening of the myocardial sarcolemmal versus mitochondrial ATP-gated potassium channel. *J Pharmacol Exp Ther*. 2004; 309(2):554–559. [PubMed: 14747611]
157. Wirth KJ, Rosenstein B, Uhde J, Englert HC, Busch AE, Scholkens BA. ATP-sensitive potassium channel blocker HMR 1883 reduces mortality and ischemia-associated electrocardiographic changes in pigs with coronary occlusion. *J Pharmacol Exp Ther*. 1999; 291(2):474–481. [PubMed: 10525061]
158. Cacciapuoti F, Spiezia R, Bianchi U, Lama D, D'Avino M, Varricchio M. Effectiveness of glibenclamide on myocardial ischemic ventricular arrhythmias in non-insulin-dependent diabetes mellitus. *Am J Cardiol*. 1991; 67(9):843–847. [PubMed: 1707221]
159. Aronson D, Mittleman MA, Burger AJ. Effects of sulfonylurea hypoglycemic agents and adenosine triphosphate dependent potassium channel antagonists on ventricular arrhythmias in patients with decompensated heart failure. *Pacing Clin Electrophysiol*. 2003; 26(5):1254–1261. [PubMed: 12765455]
160. Lomuscio A, Vergani D, Marano L, Castagnone M, Fiorentini C. Effects of glibenclamide on ventricular fibrillation in non-insulin-dependent diabetics with acute myocardial infarction. *Coron Artery Dis*. 1994; 5(9):767–771. [PubMed: 7858767]
161. Sasaki N, Sato T, Marban E, O'Rourke B. ATP consumption by uncoupled mitochondria activates sarcolemmal K_{ATP} channels in cardiac myocytes. *Am J Physiol Heart Circ Physiol*. 2001; 280(4):H1882–1888. [PubMed: 11247805]
162. Weiss JN, Lamp ST. Glycolysis preferentially inhibits ATP-sensitive K⁺ channels in isolated guinea pig cardiac myocytes. *Science*. 1987; 238(4823):67–69. [PubMed: 2443972]
163. Jovanovic S, Du Q, Crawford RM, Budas GR, Stagliar I, Jovanovic A. Glyceraldehyde 3-phosphate dehydrogenase serves as an accessory protein of the cardiac sarcolemmal K(ATP) channel. *EMBO Rep*. 2005; 6(9):848–852. [PubMed: 16082386]
164. Dhar-Chowdhury P, Harrell MD, Han SY, Jankowska D, Parachuru L, Morrissey A, Srivastava S, Liu W, Malester B, Yoshida H, Coetzee WA. The glycolytic enzymes, glyceraldehyde-3-phosphate dehydrogenase, triose-phosphate isomerase, and pyruvate kinase are components of the K(ATP) channel macromolecular complex and regulate its function. *J Biol Chem*. 2005; 280(46):38464–38470. [PubMed: 16170200]
165. Smith JM, Wahler GM. ATP-sensitive potassium channels are altered in ventricular myocytes from diabetic rats. *Mol Cell Biochem*. 1996; 158(1):43–51. [PubMed: 8791283]
166. Davies JK, Wells DJ, Liu K, Whitrow HR, Daniel TD, Grignani R, Lygate CA, Schneider JE, Noel G, Watkins H, Carling D. Characterization of the role of gamma2 R531G mutation in AMP-activated protein kinase in cardiac hypertrophy and Wolff-Parkinson-White syndrome. *Am J Physiol Heart Circ Physiol*. 2006; 290(5):H1942–1951. [PubMed: 16339829]
167. Sidhu JS, Rajawat YS, Rami TG, Gollob MH, Wang Z, Yuan R, Marian AJ, DeMayo FJ, Weilbacher D, Taffet GE, Davies JK, Carling D, Khoury DS, Roberts R. Transgenic mouse

model of ventricular preexcitation and atrioventricular reentrant tachycardia induced by an AMP-activated protein kinase loss-of-function mutation responsible for Wolff-Parkinson-White syndrome. *Circulation*. 2005; 111(1):21–29. [PubMed: 15611370]

168. Bonnet D, Martin D, Pascale De L, Villain E, Jouvet P, Rabier D, Brivet M, Saudubray JM. Arrhythmias and conduction defects as presenting symptoms of fatty acid oxidation disorders in children. *Circulation*. 1999; 100(22):2248–2253. [PubMed: 10577999]
169. Chiu HC, Kovacs A, Blanton RM, Han X, Courtois M, Weinheimer CJ, Yamada KA, Brunet S, Xu H, Nerbonne JM, Welch MJ, Fettig NM, Sharp TL, Sambandam N, Olson KM, Ory DS, Schaffer JE. Transgenic expression of fatty acid transport protein 1 in the heart causes lipotoxic cardiomyopathy. *Circ Res*. 2005; 96(2):225–233. [PubMed: 15618539]
170. Janse MJ, Wit AL. Electrophysiological mechanisms of ventricular arrhythmias resulting from myocardial ischemia and infarction. *Physiol Rev*. 1989; 69(4):1049–1169. [PubMed: 2678165]
171. Fozzard HA, Makielski JC. The electrophysiology of acute myocardial ischemia. *Annu Rev Med*. 1985; 36:275–284. [PubMed: 2581499]
172. Janse MJ. Electrophysiological changes in heart failure and their relationship to arrhythmogenesis. *Cardiovasc Res*. 2004; 61(2):208–217. [PubMed: 14736537]
173. Liu M, Yang KC, Dudley SC Jr. Cardiac sodium channel mutations: why so many phenotypes? *Nat Rev Cardiol*. 2014
174. Song Y, Shryock JC, Wagner S, Maier LS, Belardinelli L. Blocking late sodium current reduces hydrogen peroxide-induced arrhythmogenic activity and contractile dysfunction. *J Pharmacol Exp Ther*. 2006; 318(1):214–222. [PubMed: 16565163]
175. Shang LL, Sanyal S, Pfahnl AE, Jiao Z, Allen J, Liu H, Dudley SC Jr. NF-kappaB-dependent transcriptional regulation of the cardiac scn5a sodium channel by angiotensin II. *Am J Physiol Cell Physiol*. 2008; 294(1):C372–379. [PubMed: 18032528]
176. Gao G, Xie A, Zhang J, Herman AM, Jeong EM, Gu L, Liu M, Yang KC, Kamp TJ, Dudley SC. Unfolded protein response regulates cardiac sodium current in systolic human heart failure. *Circ Arrhythm Electrophysiol*. 2013; 6(5):1018–1024. [PubMed: 24036084]
177. Kassmann M, Hansel A, Leipold E, Birkenbeil J, Lu SQ, Hoshi T, Heinemann SH. Oxidation of multiple methionine residues impairs rapid sodium channel inactivation. *Pflugers Arch*. 2008; 456(6):1085–1095. [PubMed: 18369661]
178. Fukuda K, Davies SS, Nakajima T, Ong BH, Kupersmidt S, Fessel J, Amarnath V, Anderson ME, Boyden PA, Viswanathan PC, Roberts LJ 2nd, Balser JR. Oxidative mediated lipid peroxidation recapitulates proarrhythmic effects on cardiac sodium channels. *Circ Res*. 2005; 97(12):1262–1269. [PubMed: 16284182]
179. Liu M, Sanyal S, Gao G, Gurung IS, Zhu X, Gaconnet G, Kerchner LJ, Shang LL, Huang CL, Grace A, London B, Dudley SC Jr. Cardiac Na⁺ current regulation by pyridine nucleotides. *Circ Res*. 2009; 105(8):737–745. [PubMed: 19745168]
180. Depre C, Fierain L, Hue L. Activation of nitric oxide synthase by ischaemia in the perfused heart. *Cardiovasc Res*. 1997; 33(1):82–87. [PubMed: 9059531]
181. Wang P, Zweier JL. Measurement of nitric oxide and peroxynitrite generation in the postischemic heart. Evidence for peroxynitrite-mediated reperfusion injury. *J Biol Chem*. 1996; 271(46):29223–29230. [PubMed: 8910581]
182. Simon JN, Duglan D, Casadei B, Carnicer R. Nitric oxide synthase regulation of cardiac excitation-contraction coupling in health and disease. *J Mol Cell Cardiol*. 2014; 73:80–91. [PubMed: 24631761]
183. Weerateerangkul P, Chattipakorn S, Chattipakorn N. Roles of the nitric oxide signaling pathway in cardiac ischemic preconditioning against myocardial ischemia-reperfusion injury. *Med Sci Monit*. 2011; 17(2):RA44–52. [PubMed: 21278703]
184. Ahern GP, Hsu SF, Klyachko VA, Jackson MB. Induction of persistent sodium current by exogenous and endogenous nitric oxide. *J Biol Chem*. 2000; 275(37):28810–28815. [PubMed: 10833522]
185. Hammarstrom AK, Gage PW. Nitric oxide increases persistent sodium current in rat hippocampal neurons. *J Physiol*. 1999; 520(Pt 2):451–461. [PubMed: 10523414]

186. Ahmmed GU, Xu Y, Hong Dong P, Zhang Z, Eiserich J, Chiamvimonvat N. Nitric oxide modulates cardiac Na^+ channel via protein kinase A and protein kinase G. *Circ Res.* 2001; 89(11):1005–1013. [PubMed: 11717157]
187. Gautier M, Zhang H, Fearon IM. Peroxynitrite formation mediates LPC-induced augmentation of cardiac late sodium currents. *J Mol Cell Cardiol.* 2008; 44(2):241–251. [PubMed: 17961592]
188. Goldhaber JL, Ji S, Lamp ST, Weiss JN. Effects of exogenous free radicals on electromechanical function and metabolism in isolated rabbit and guinea pig ventricle. Implications for ischemia and reperfusion injury. *J Clin Invest.* 1989; 83(6):1800–1809. [PubMed: 2723059]
189. Viola HM, Arthur PG, Hool LC. Transient exposure to hydrogen peroxide causes an increase in mitochondria-derived superoxide as a result of sustained alteration in L-type Ca^{2+} channel function in the absence of apoptosis in ventricular myocytes. *Circ Res.* 2007; 100(7):1036–1044. [PubMed: 17347474]
190. Fearon IM. OxLDL enhances L-type Ca^{2+} currents via lysophosphatidylcholine-induced mitochondrial reactive oxygen species (ROS) production. *Cardiovasc Res.* 2006; 69(4):855–864. [PubMed: 16412401]
191. Coetzee WA, Opie LH. Effects of oxygen free radicals on isolated cardiac myocytes from guinea-pig ventricle: electrophysiological studies. *J Mol Cell Cardiol.* 1992; 24(6):651–663. [PubMed: 1518081]
192. Hammerschmidt S, Wahn H. The effect of the oxidant hypochlorous acid on the L-type calcium current in isolated ventricular cardiac myocytes. *J Mol Cell Cardiol.* 1998; 30(9):1855–1867. [PubMed: 9769240]
193. Kawakami M, Okabe E. Superoxide anion radical-triggered Ca^{2+} release from cardiac sarcoplasmic reticulum through ryanodine receptor Ca^{2+} channel. *Mol Pharmacol.* 1998; 53(3):497–503. [PubMed: 9495817]
194. Yan Y, Liu J, Wei C, Li K, Xie W, Wang Y, Cheng H. Bidirectional regulation of Ca^{2+} sparks by mitochondria-derived reactive oxygen species in cardiac myocytes. *Cardiovasc Res.* 2008; 77(2):432–441. [PubMed: 18006452]
195. Scherer NM, Deamer DW. Oxidative stress impairs the function of sarcoplasmic reticulum by oxidation of sulfhydryl groups in the Ca^{2+} -ATPase. *Arch Biochem Biophys.* 1986; 246(2):589–601. [PubMed: 2939799]
196. Kukreja RC, Kearns AA, Zweier JL, Kuppusamy P, Hess ML. Singlet oxygen interaction with Ca^{2+} -ATPase of cardiac sarcoplasmic reticulum. *Circ Res.* 1991; 69(4):1003–1014. [PubMed: 1657435]
197. Xu KY, Zweier JL, Becker LC. Hydroxyl radical inhibits sarcoplasmic reticulum Ca^{2+} -ATPase function by direct attack on the ATP binding site. *Circ Res.* 1997; 80(1):76–81. [PubMed: 8978325]
198. Eigel BN, Gursahani H, Hadley RW. ROS are required for rapid reactivation of $\text{Na}^+/\text{Ca}^{2+}$ exchanger in hypoxic reoxygenated guinea pig ventricular myocytes. *Am J Physiol Heart Circ Physiol.* 2004; 286(3):H955–963. [PubMed: 14592940]
199. Goldhaber JL. Free radicals enhance $\text{Na}^+/\text{Ca}^{2+}$ exchange in ventricular myocytes. *Am J Physiol.* 1996; 271(3 Pt 2):H823–833. [PubMed: 8853314]
200. Rokita AG, Anderson ME. New therapeutic targets in cardiology: arrhythmias and Ca^{2+} /calmodulin-dependent kinase II (CaMKII). *Circulation.* 2012; 126(17):2125–2139. [PubMed: 23091085]
201. Wu Y, MacMillan LB, McNeill RB, Colbran RJ, Anderson ME. CaM kinase augments cardiac L-type Ca^{2+} current: a cellular mechanism for long Q-T arrhythmias. *Am J Physiol.* 1999; 276(6 Pt 2):H2168–2178. [PubMed: 10362701]
202. Di Lisa F, Blank PS, Colonna R, Gambassi G, Silverman HS, Stern MD, Hansford RG. Mitochondrial membrane potential in single living adult rat cardiac myocytes exposed to anoxia or metabolic inhibition. *J Physiol.* 1995; 486 (Pt 1):1–13. [PubMed: 7562625]
203. Griffiths EJ, Ocampo CJ, Savage JS, Rutter GA, Hansford RG, Stern MD, Silverman HS. Mitochondrial calcium transporting pathways during hypoxia and reoxygenation in single rat cardiac myocytes. *Cardiovasc Res.* 1998; 39(2):423–433. [PubMed: 9798527]

204. Perez-Campo R, Lopez-Torres M, Cadenas S, Rojas C, Barja G. The rate of free radical production as a determinant of the rate of aging: evidence from the comparative approach. *J Comp Physiol B*. 1998; 168(3):149–158. [PubMed: 9591361]
205. Sohal RS, Allen RG. Relationship between metabolic rate, free radicals, differentiation and aging: a unified theory. *Basic Life Sci*. 1985; 35:75–104. [PubMed: 4062824]
206. Cleeter MW, Cooper JM, Darley-USmar VM, Moncada S, Schapira AH. Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. Implications for neurodegenerative diseases. *FEBS Lett*. 1994; 345(1):50–54. [PubMed: 8194600]
207. Ott M, Robertson JD, Gogvadze V, Zhivotovsky B, Orrenius S. Cytochrome c release from mitochondria proceeds by a two-step process. *Proc Natl Acad Sci U S A*. 2002; 99(3):1259–1263. [PubMed: 11818574]
208. Grijalba MT, Vercesi AE, Schreier S. Ca^{2+} -induced increased lipid packing and domain formation in submitochondrial particles. A possible early step in the mechanism of Ca^{2+} -stimulated generation of reactive oxygen species by the respiratory chain. *Biochemistry*. 1999; 38(40):13279–13287. [PubMed: 10529202]
209. Dedkova EN, Blatter LA. Characteristics and function of cardiac mitochondrial nitric oxide synthase. *J Physiol*. 2009; 587(Pt 4):851–872. [PubMed: 19103678]
210. Feissner RF, Skalska J, Gaum WE, Sheu SS. Crosstalk signaling between mitochondrial Ca^{2+} and ROS. *Front Biosci (Landmark Ed)*. 2009; 14:1197–1218. [PubMed: 19273125]
211. Hu H, Chiamvimonvat N, Yamagishi T, Marban E. Direct inhibition of expressed cardiac L-type Ca^{2+} channels by S-nitrosothiol nitric oxide donors. *Circ Res*. 1997; 81(5):742–752. [PubMed: 9351448]
212. Sun J, Picht E, Ginsburg KS, Bers DM, Steenbergen C, Murphy E. Hypercontractile female hearts exhibit increased S-nitrosylation of the L-type Ca^{2+} channel α_1 subunit and reduced ischemia/reperfusion injury. *Circ Res*. 2006; 98(3):403–411. [PubMed: 16397145]
213. Wang H, Kohr MJ, Wheeler DG, Ziolo MT. Endothelial nitric oxide synthase decreases beta-adrenergic responsiveness via inhibition of the L-type Ca^{2+} current. *Am J Physiol Heart Circ Physiol*. 2008; 294(3):H1473–1480. [PubMed: 18203845]
214. Sun J, Yamaguchi N, Xu L, Eu JP, Stamler JS, Meissner G. Regulation of the cardiac muscle ryanodine receptor by O_2 tension and S-nitrosoglutathione. *Biochemistry*. 2008; 47(52):13985–13990. [PubMed: 19053230]
215. Lokuta AJ, Maertz NA, Meethal SV, Potter KT, Kamp TJ, Valdivia HH, Haworth RA. Increased nitration of sarcoplasmic reticulum Ca^{2+} -ATPase in human heart failure. *Circulation*. 2005; 111(8):988–995. [PubMed: 15710754]
216. Cerbai E, Ambrosio G, Porciatti F, Chiariello M, Giotti A, Mugelli A. Cellular electrophysiological basis for oxygen radical-induced arrhythmias. A patch-clamp study in guinea pig ventricular myocytes. *Circulation*. 1991; 84(4):1773–1782. [PubMed: 1655304]
217. Liang H, Li X, Li S, Zheng MQ, Rozanski GJ. Oxidoreductase regulation of Kv currents in rat ventricle. *J Mol Cell Cardiol*. 2008; 44(6):1062–1071. [PubMed: 18455732]
218. Li X, Tang K, Xie B, Li S, Rozanski GJ. Regulation of Kv4 channel expression in failing rat heart by the thioredoxin system. *Am J Physiol Heart Circ Physiol*. 2008; 295(1):H416–424. [PubMed: 18515646]
219. Li S, Li X, Li YL, Shao CH, Bidasee KR, Rozanski GJ. Insulin regulation of glutathione and contractile phenotype in diabetic rat ventricular myocytes. *Am J Physiol Heart Circ Physiol*. 2007; 292(3):H1619–1629. [PubMed: 17056675]
220. Chiarugi P. PTPs versus PTKs: the redox side of the coin. *Free Radic Res*. 2005; 39(4):353–364. [PubMed: 16028361]
221. Nakamura TY, Coetzee WA, Vega-Saenz De Miera E, Artman M, Rudy B. Modulation of Kv4 channels, key components of rat ventricular transient outward K^+ current, by PKC. *Am J Physiol*. 1997; 273(4 Pt 2):H1775–1786. [PubMed: 9362243]
222. Li GR, Feng J, Wang Z, Fermini B, Nattel S. Adrenergic modulation of ultrarapid delayed rectifier K^+ current in human atrial myocytes. *Circ Res*. 1996; 78(5):903–915. [PubMed: 8620611]

223. Gomez R, Nunez L, Vaquero M, Amoros I, Barana A, de Prada T, Macaya C, Maroto L, Rodriguez E, Caballero R, Lopez-Farre A, Tamargo J, Delpon E. Nitric oxide inhibits Kv_{4.3} and human cardiac transient outward potassium current (I_{to1}). *Cardiovasc Res*. 2008; 80(3):375–384. [PubMed: 18678642]
224. Nunez L, Vaquero M, Gomez R, Caballero R, Mateos-Caceres P, Macaya C, Iriepa I, Galvez E, Lopez-Farre A, Tamargo J, Delpon E. Nitric oxide blocks hKv1.5 channels by S-nitrosylation and by a cyclic GMP-dependent mechanism. *Cardiovasc Res*. 2006; 72(1):80–89. [PubMed: 16876149]
225. Ryu SY, Lee SH, Ho WK. Generation of metabolic oscillations by mitoK_{ATP} and ATP synthase during simulated ischemia in ventricular myocytes. *J Mol Cell Cardiol*. 2005; 39(6):874–881. [PubMed: 16242144]
226. Zorov DB, Filburn CR, Klotz LO, Zweier JL, Sollott SJ. Reactive oxygen species (ROS)-induced ROS release: a new phenomenon accompanying induction of the mitochondrial permeability transition in cardiac myocytes. *J Exp Med*. 2000; 192(7):1001–1014. [PubMed: 11015441]
227. Brown DA, Aon MA, Akar FG, Liu T, Sorrairain N, O'Rourke B. Effects of 4'-chlorodiazepam on cellular excitation-contraction coupling and ischaemia-reperfusion injury in rabbit heart. *Cardiovasc Res*. 2008; 79(1):141–149. [PubMed: 18304929]
228. Brown DA, Aon MA, Frasier CR, Sloan RC, Maloney AH, Anderson EJ, O'Rourke B. Cardiac arrhythmias induced by glutathione oxidation can be inhibited by preventing mitochondrial depolarization. *J Mol Cell Cardiol*. 2010; 48(4):673–679. [PubMed: 19962380]
229. O'Rourke B. Evidence for mitochondrial K⁺ channels and their role in cardioprotection. *Circ Res*. 2004; 94(4):420–432. [PubMed: 15001541]
230. Garlid KD, Dos Santos P, Xie ZJ, Costa AD, Paucek P. Mitochondrial potassium transport: the role of the mitochondrial ATP-sensitive K⁺ channel in cardiac function and cardioprotection. *Biochim Biophys Acta*. 2003; 1606(1–3):1–21. [PubMed: 14507424]
231. Murata M, Akao M, O'Rourke B, Marban E. Mitochondrial ATP-sensitive potassium channels attenuate matrix Ca²⁺ overload during simulated ischemia and reperfusion: possible mechanism of cardioprotection. *Circ Res*. 2001; 89(10):891–898. [PubMed: 11701616]
232. Wang L, Cherednichenko G, Hernandez L, Halow J, Camacho SA, Figueredo V, Schaefer S. Preconditioning limits mitochondrial Ca²⁺ during ischemia in rat hearts: role of K(ATP) channels. *Am J Physiol Heart Circ Physiol*. 2001; 280(5):H2321–2328. [PubMed: 11299237]
233. Oldenburg O, Cohen MV, Downey JM. Mitochondrial K_{ATP} channels in preconditioning. *J Mol Cell Cardiol*. 2003; 35(6):569–575. [PubMed: 12788373]
234. Forbes RA, Steenbergen C, Murphy E. Diazoxide-induced cardioprotection requires signaling through a redox-sensitive mechanism. *Circ Res*. 2001; 88(8):802–809. [PubMed: 11325872]
235. Sasaki N, Sato T, Ohler A, O'Rourke B, Marban E. Activation of mitochondrial ATP-dependent potassium channels by nitric oxide. *Circulation*. 2000; 101(4):439–445. [PubMed: 10653837]
236. Bolli R, Manchikalapudi S, Tang XL, Takano H, Qiu Y, Guo Y, Zhang Q, Jadoon AK. The protective effect of late preconditioning against myocardial stunning in conscious rabbits is mediated by nitric oxide synthase. Evidence that nitric oxide acts both as a trigger and as a mediator of the late phase of ischemic preconditioning. *Circ Res*. 1997; 81(6):1094–1107. [PubMed: 9400391]
237. Bolli R, Bhatti ZA, Tang XL, Qiu Y, Zhang Q, Guo Y, Jadoon AK. Evidence that late preconditioning against myocardial stunning in conscious rabbits is triggered by the generation of nitric oxide. *Circ Res*. 1997; 81(1):42–52. [PubMed: 9201026]
238. Vegh A, Parratt JR. The role of mitochondrial K_{ATP} channels in antiarrhythmic effects of ischaemic preconditioning in dogs. *Br J Pharmacol*. 2002; 137(7):1107–1115. [PubMed: 12429584]
239. Headrick JP, Willems L, Ashton KJ, Holmgren K, Peart J, Matherne GP. Ischaemic tolerance in aged mouse myocardium: the role of adenosine and effects of A1 adenosine receptor overexpression. *J Physiol*. 2003; 549(Pt 3):823–833. [PubMed: 12717009]
240. Das B, Sarkar C. Is the sarcolemmal or mitochondrial K_{ATP} channel activation important in the antiarrhythmic and cardioprotective effects during acute ischemia/reperfusion in the intact anesthetized rabbit model? *Life Sci*. 2005; 77(11):1226–1248. [PubMed: 15964023]

241. Lo CW. Role of gap junctions in cardiac conduction and development: insights from the connexin knockout mice. *Circ Res.* 2000; 87(5):346–348. [PubMed: 10969030]
242. Litchenberg WH, Norman LW, Holwell AK, Martin KL, Hewett KW, Gourdie RG. The rate and anisotropy of impulse propagation in the postnatal terminal crest are correlated with remodeling of Cx43 gap junction pattern. *Cardiovasc Res.* 2000; 45(2):379–387. [PubMed: 10728358]
243. Kaprielian RR, Gunning M, Dupont E, Sheppard MN, Rothery SM, Underwood R, Pennell DJ, Fox K, Pepper J, Poole-Wilson PA, Severs NJ. Downregulation of immunodetectable connexin43 and decreased gap junction size in the pathogenesis of chronic hibernation in the human left ventricle. *Circulation.* 1998; 97(7):651–660. [PubMed: 9495300]
244. Peters NS, Green CR, Poole-Wilson PA, Severs NJ. Reduced content of connexin43 gap junctions in ventricular myocardium from hypertrophied and ischemic human hearts. *Circulation.* 1993; 88(3):864–875. [PubMed: 8394786]
245. Dupont E, Matsushita T, Kaba RA, Vozzi C, Coppen SR, Khan N, Kaprielian R, Yacoub MH, Severs NJ. Altered connexin expression in human congestive heart failure. *J Mol Cell Cardiol.* 2001; 33(2):359–371. [PubMed: 11162139]
246. Kostin S, Rieger M, Dammer S, Hein S, Richter M, Klovekorn WP, Bauer EP, Schaper J. Gap junction remodeling and altered connexin43 expression in the failing human heart. *Mol Cell Biochem.* 2003; 242(1–2):135–144. [PubMed: 12619876]
247. Jongsma HJ, Wilders R. Gap junctions in cardiovascular disease. *Circ Res.* 2000; 86(12):1193–1197. [PubMed: 10864907]
248. Gutstein DE, Morley GE, Tamaddon H, Vaidya D, Schneider MD, Chen J, Chien KR, Stuhlmann H, Fishman GI. Conduction slowing and sudden arrhythmic death in mice with cardiac-restricted inactivation of connexin43. *Circ Res.* 2001; 88(3):333–339. [PubMed: 11179202]
249. Efimov IR. Connections, connections, connexins: towards systems biology paradigm of cardiac arrhythmia. *J Mol Cell Cardiol.* 2006; 41(6):949–951. [PubMed: 17030039]
250. van Rijen HV, van Veen TA, Gros D, Wilders R, de Bakker JM. Connexins and cardiac arrhythmias. *Adv Cardiol.* 2006; 42:150–160. [PubMed: 16646589]
251. Ikram H. The renin-angiotensin-aldosterone system and cardiac ischaemia. *Heart.* 1996; 76(3 Suppl 3):60–67. [PubMed: 8983666]
252. Santos RA, Brum JM, Brosnihan KB, Ferrario CM. The renin-angiotensin system during acute myocardial ischemia in dogs. *Hypertension.* 1990; 15(2 Suppl):1121–127. [PubMed: 2404854]
253. De Mello WC. Renin-angiotensin system and cell communication in the failing heart. *Hypertension.* 1996; 27(6):1267–1272. [PubMed: 8641734]
254. De Mello WC. Is an intracellular renin-angiotensin system involved in control of cell communication in heart? *J Cardiovasc Pharmacol.* 1994; 23(4):640–646. [PubMed: 7516016]
255. Xiao HD, Fuchs S, Campbell DJ, Lewis W, Dudley SC Jr, Kasi VS, Hoit BD, Keshelava G, Zhao H, Capecci MR, Bernstein KE. Mice with cardiac-restricted angiotensin-converting enzyme (ACE) have atrial enlargement, cardiac arrhythmia, and sudden death. *Am J Pathol.* 2004; 165(3):1019–1032. [PubMed: 15331425]
256. Donoghue M, Wakimoto H, Maguire CT, Acton S, Hales P, Stagliano N, Fairchild-Huntress V, Xu J, Lorenz JN, Kadambi V, Berul CI, Breitbart RE. Heart block, ventricular tachycardia, and sudden death in ACE2 transgenic mice with downregulated connexins. *J Mol Cell Cardiol.* 2003; 35(9):1043–1053. [PubMed: 12967627]
257. Sovari AA, Iravanian S, Dolmatova E, Jiao Z, Liu H, Zandieh S, Kumar V, Wang K, Bernstein KE, Bonini MG, Duffy HS, Dudley SC. Inhibition of c-Src tyrosine kinase prevents angiotensin II-mediated connexin-43 remodeling and sudden cardiac death. *J Am Coll Cardiol.* 2011; 58(22):2332–2339. [PubMed: 22093512]
258. Iravanian S, Sovari AA, Lardin HA, Liu H, Xiao HD, Dolmatova E, Jiao Z, Harris BS, Witham EA, Gourdie RG, Duffy HS, Bernstein KE, Dudley SC Jr. Inhibition of renin-angiotensin system (RAS) reduces ventricular tachycardia risk by altering connexin43. *J Mol Med (Berl).* 2011; 89(7):677–687. [PubMed: 21553032]
259. Kieken F, Mutsaers N, Dolmatova E, Virgil K, Wit AL, Kellezi A, Hirst-Jensen BJ, Duffy HS, Sorgen PL. Structural and molecular mechanisms of gap junction remodeling in epicardial border

- zone myocytes following myocardial infarction. *Circ Res.* 2009; 104(9):1103–1112. [PubMed: 19342602]
260. Toyofuku T, Yabuki M, Otsu K, Kuzuya T, Tada M, Hori M. Functional role of c-Src in gap junctions of the cardiomyopathic heart. *Circ Res.* 1999; 85(8):672–681. [PubMed: 10521240]
261. Myung SK, Ju W, Cho B, Oh SW, Park SM, Koo BK, Park BJ. Efficacy of vitamin and antioxidant supplements in prevention of cardiovascular disease: systematic review and meta-analysis of randomised controlled trials. *BMJ.* 2013; 346:f10. [PubMed: 23335472]
262. Yang KC, Rutledge CA, Mao M, Bakhshi FR, Xie A, Liu H, Bonini MG, Patel HH, Minshall RD, Dudley SC Jr. Caveolin-1 Modulates Cardiac Gap Junction Homeostasis and Arrhythmogenicity by Regulating cSrc Tyrosine Kinase. *Circ Arrhythm Electrophysiol.* 2014; 7(4):701–710. [PubMed: 25017399]
263. Hatch F, Lancaster MK, Jones SA. Aging is a primary risk factor for cardiac arrhythmias: disruption of intracellular Ca^{2+} regulation as a key suspect. *Expert Rev Cardiovasc Ther.* 2011; 9(8):1059–1067. [PubMed: 21878050]
264. Ludka O, Konecny T, Somers V. Sleep apnea, cardiac arrhythmias, and sudden death. *Tex Heart Inst J.* 2011; 38(4):340–343. [PubMed: 21841855]
265. Roberts PR, Green D. Arrhythmias in chronic kidney disease. *Heart.* 2011; 97(9):766–773. [PubMed: 21474617]
266. Biernacka A, Frangogiannis NG. Aging and Cardiac Fibrosis. *Aging Dis.* 2011; 2(2):158–173. [PubMed: 21837283]
267. Asbun J, Villarreal FJ. The pathogenesis of myocardial fibrosis in the setting of diabetic cardiomyopathy. *J Am Coll Cardiol.* 2006; 47(4):693–700. [PubMed: 16487830]
268. Xie C, Kauffman J, Akar FG. Functional crosstalk between the mitochondrial PTP and K_{ATP} channels determine arrhythmic vulnerability to oxidative stress. *Front Physiol.* 2014; 5:264. [PubMed: 25076913]

Figure 1. Action potential waveform and major ion channels/transporters in human ventricular cardiac myocytes

The action potential (AP) waveform begins with the activation of Na^+ channels (I_{Na}), leading to the rapid depolarization (phase 0), followed by inward repolarizing transient outward K^+ currents (I_{to}) in phase 1, forming the notch of the AP waveform. The balance between the inward L-type Ca^{2+} current (I_{CaL}) and outward delayed rectifier K^+ currents (I_K) contributes to the plateau (phase 2) of the AP waveform. I_K continues to repolarize the membrane potential (phase 3) and the inwardly rectifying K^+ currents (I_{K1}) contributes to the later part of phase 3 repolarization and the maintenance of resting membrane potential (phase 4). During the AP, Ca^{2+} influx via I_{CaL} triggers the release of Ca^{2+} ions from the sarcoplasmic reticulum (SR) via ryanodine receptor 2 (RyR2), coined “ Ca^{2+} -induced Ca^{2+} -release.” During the diastolic phase, sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) retrieves cytosolic Ca^{2+} to SR, and Na^+/Ca^{2+} exchanger (NCX) extrudes Ca^{2+} from the cell, bringing cytosolic $[Ca^{2+}]$ back to baseline. Sarcolemmal K_{ATP} channels (sarcoK_{ATP}), gated by intracellular ATP/ADP levels and acidosis, does not contribute to AP waveform under

normal circumstances, but play an important role in regulating cellular metabolism and electrophysiological responses to metabolic and oxidative stresses. Na^+/K^+ ATPase consumes ATP to pump Na^+ outside of the cell in exchange for K^+ , which is critical for the maintenance of Na^+ and K^+ gradients across the plasma membrane.

Figure 2. Oxidative phosphorylation and ROS production in mitochondria

The diagrams of the mitochondrial inner membrane show key components of the electron transport chain (ETC) above, and channels and transporters (below). Reducing equivalents NADH and FADH₂ produced from the TCA cycle feed electrons to the ETC along the mitochondrial inner membrane. The electrons flow through the ETC with the following sequence: complex I & II → coenzyme Q [Q] → complex III → cytochrome C [Cyt C] → complex IV → O₂, during which coupled redox reactions drive H⁺ across the inner membrane, forming the proton gradient and the negative mitochondrial membrane potential, Ψ_m (~-150 to -180 mV). The free energy stored in the proton gradient and Ψ_m then drive H⁺ through the mitochondrial ATP synthase (complex V), converting ADP to ATP. An estimated 0.1–1% of the electrons leak prematurely to O₂ at complexes I, II or III, resulting in the formation of superoxide (O₂^{•-}). Multiple important mitochondrial channels located on the inner membrane including mitochondrial K_{ATP} channels (mitoK_{ATP}), the mitochondrial Na⁺/Ca²⁺ exchanger (mitoNCX), the inner membrane anion channel (IMAC), the permeability transition pore (PTP) and the mitochondrial calcium uniporter (MCU) also contribute to the regulation of mitochondrial function, myocardial ROS and cellular cation homeostasis.

Figure 3. Major ROS generation pathways in cardiac myocytes

Mitochondria represent the predominant source of ROS in cardiac myocytes, producing superoxide ($\text{O}_2^{\bullet-}$) by the premature leak of electrons from the respiratory chain at complexes I, II or III. Mitochondrial MnSOD can dismutate $\text{O}_2^{\bullet-}$ to hydrogen peroxide (H_2O_2), which can be broken down by transition metals such as Fe^{2+} and converted to hydroxyl radicals ($\bullet\text{OH}$). Superoxide can also be generated by the reduction of O_2 by free electrons released from (1) NAD(P)H oxidases, which oxidize NADH or NADPH to form NAD^+ or NADP^+ , (2) xanthine oxidase, which catalyzes the conversion of hypoxanthine to xanthine or xanthine to uric acid, (3) endothelial nitric oxide synthase (eNOS), which switches from a coupled state (producing $\text{NO}^\bullet$) to an uncoupled state (producing $\text{O}_2^{\bullet-}$) under conditions such as the reduction in L-arginine, the deficiency of cofactor tetrahydrobiopterin (BH_4), or increased glutathionylation of eNOS. Superoxide can also interact with preformed $\text{NO}^\bullet$ and generate peroxynitrite ONOO^- .

Figure 4. Effects of metabolic stresses on cardiac ion channel/transporter function

Schematic illustration of the impact of acute (ischemia/hypoxia) and chronic (heart failure and diabetes) metabolic stresses on cardiac ion channel/transporter function and the electrophysiological consequences. Acidosis during acute ischemia leads to opening of $\text{sarck}_{\text{ATP}}$ channels, increased activity of NHE, and increased late I_{Na} . ATP depletion during ischemia contributes to the opening of $\text{sarck}_{\text{ATP}}$ channels, as well as impairs the function of Na/K ATPase. Other electrophysiological effects from ischemia include the reduction of peak I_{Na} , increased connexin conduction, and reduced RyR2 activity. Reduced RyR2 activity during acute ischemia is rapidly reversed upon reperfusion, contributing to the spontaneous waves of Ca^{2+} release and DADs. A few examples of electrophysiological

impact from chronic metabolic stresses associated with heart failure and diabetes are also illustrated: heart failure reduces peak I_{Na} and multiple K_v (I_{to} , I_K) currents. Diabetes is also associated with reduction in K_v currents. The increase in $sarK_{ATP}$ currents lead to APD shortening, whereas reduced K_v currents and increased late I_{Na} lead to prolonged APD and EADs. Finally, reduced Na/K ATPase activity, increased RyR2 activity and increased gap junction conduction during ischemia predispose to arrhythmogenic DADs.

Figure 5. Effects of increased oxidative stress on cardiac ion channel/transporter function
 Schematic figure to demonstrate the impact of increased oxidative stress on cardiac ion channel/transporter function. Increased ROS enhance the activity of RyR2 and sarcoK_{ATP} channels and increase late I_{Na}. Excessive oxidative stress reduces peak I_{Na} and repolarizing K⁺ currents (I_{to}, I_K and I_{K1}) and leads to reduced gap junction protein Cx43 and reduced SERCA activity. Increased ROS inhibit normal operation of NCX (Na⁺ in, Ca²⁺ out) and promote the reverse mode of NCX activity (Na⁺ out, Ca²⁺ in). The increase in sarcoK_{ATP} currents lead to APD shortening, whereas reduced K⁺ currents and increased late I_{Na} lead to prolonged APD and EADs. Increased RyR2 activity, reduced SERCA function, and reverse mode NCX activity lead to cytosolic Ca²⁺ overload and predispose to DADs.

Table 1

Effects of metabolic changes on cardiac ion channel/transporter function and arrhythmogenicity

Channel/Transporter effects	Metabolic changes	Effects on electrical/ionic homeostasis	Pro-arrhythmic mechanism
Na ⁺ /K ⁺ ATPase ↓	Ischemia/hypoxia	Na ⁺ overload	Ca ²⁺ overload and DAD
Cx hemichannel ↑	Ischemia	Na ⁺ overload	Ca ²⁺ overload and DAD
Peak I _{Na} ↓	Ischemia/heart failure	↓ Na ⁺ influx	Slow conduction
Late I _{Na} ↑	Ischemia/hypoxia acidosis, ↑ LPC AMPK mutation	↑ Na ⁺ influx, prolonged APD	EAD
Kv ↓	Diabetes, Heart failure	↓ K ⁺ influx, prolonged APD	EAD
Kv ↑	Insulin treatment in diabetic heart, PI3Kα activation, Exercise training	↑ K ⁺ channel expression	Protective
I _{KATP} ↑	Ischemia	↑ K ⁺ influx, shortened APD	Current sink, slow conduction
RyR2	↓ during ischemia; ↑ upon reperfusion	SR Ca ²⁺ load ↑, spontaneous Ca ²⁺ waves ↑	Ca ²⁺ transient/action potential alternans

Cx: connexin; Peak I_{Na}: peak Na current ; Late I_{Na}: late Na current; Kv: voltage-gated K current; I_{KATP}: ATP-sensitive K⁺ current; RyR2: ryanodine receptor 2; APD: action potential duration; SR: sarcoplasmic reticulum; EAD: early after-depolarization; DAD: delayed after-depolarization; LPC: lysophosphatidylcholine.

Table 2

Effects of increased oxidative stress on cardiac ion channel/transporter function and arrhythmogenicity

Channel/Transporter	Effects of ROS	Effects on electrical/ionic homeostasis	Pro-arrhythmic mechanism
Peak I_{Na}	↓	↓ Na^+ influx	Slow conduction
Late I_{Na}	↑	↑ Na^+ influx, APD prolonged	EAD
I_{to}	↓	↓ K^+ influx, APD prolonged	EAD
I_K	↓	↓ K^+ influx, APD prolonged	EAD
I_{K1}	↓	↓ K^+ influx, APD prolonged	EAD
I_{KATP}	↑	↑ K^+ influx, APD shortened	Current sink, slow conduction
NCX	Reverse mode	Ca^{2+} overload	DAD
Cx43	↓	Impaired gap junction function	Slow conduction
SERCA	↓	Ca^{2+} overload	DAD
RyR2	↑	Ca^{2+} overload	DAD
mitoNCX	Reverse mode	Ca^{2+} overload	DAD
mito K_{ATP}	↑	↑ Mitochondrial K^+ influx	Protective, ischemic- preconditioning

Peak I_{Na} : peak Na^+ current; Late I_{Na} : late Na^+ current; I_{to} : transient outward K^+ current; I_K : delayed rectifier K^+ current; I_{K1} : inwardly rectifying K^+ current; I_{KATP} : ATP-sensitive K^+ current; NCX: Na^+/Ca^{2+} exchanger; Cx43: connexin 43; SERCA: sarco/endoplasmic reticulum Ca^{2+} -ATPase; RyR2: ryanodine receptor 2; mitoNCX: mitochondrial Na^+/Ca^{2+} exchanger; mito K_{ATP} : mitochondrial ATP-sensitive K^+ current; APD: action potential duration; EAD: early after-depolarization; DAD: delayed after-depolarization.

Table 3

Potential novel therapeutics against ventricular arrhythmias and SCDs by targeting metabolic and oxidative stress

Type of therapeutics	Mechanism	Effects on channel function/electrophysiology
NHE inhibitor ¹¹¹	Inhibit NHE activation- induced Na ⁺ and Ca ²⁺ overload	Prevent ischemia/acidosis-induced Ca ²⁺ overload
PI3K α signaling pathway activator ^{145, 146}	$\uparrow$ K ⁺ channels transcriptionally	Restoring K ⁺ currents with HF, cardiac hypertrophy and DM
MCU inhibitor ¹⁰⁵	Inhibit mitochondrial Ca ²⁺ uptake	Prevent mitochondrial Ca ²⁺ overload and subsequent mitochondrial dysfunction
PTP inhibitor ²⁶⁸	Prevent ROS-induced ψ_m depolarization	Prevent mitochondrial ROS-induced ROS release and subsequent Ca ²⁺ overload
IMAC inhibitor ^{62, 227, 228}	Prevent ROS-induced ψ_m depolarization $\uparrow$ Mitochondrial K ⁺	Prevent mitochondrial ROS-induced ROS release and subsequent Ca ²⁺ overload
mitoK _{ATP} opener ²³⁰²²⁹	influx, prevents ψ_m depolarization	Stabilize ψ_m and maintain mitochondrial function
Mitochondria-targeted anti-oxidants ^{35, 57, 262}	$\downarrow$ mitochondrial ROS	Alleviate Cx43 and peak I _{Na} down- regulation with increased mitochondrial ROS
CaMKII δ inhibitor ¹²⁴	Prevent ROS-induced late I _{Na}	Prevent ROS-induced late I _{Na} and EAD

NHE: Na⁺-H⁺ exchanger; PI3K α : phosphoinositide 3-kinase α ; MCU: mitochondrial calcium uniporter; PTP: mitochondrial permeability transition pore; IMAC: mitochondrial inner membrane anion channel; ψ_m : mitochondrial membrane potential