

Sudden Cardiac Death Compendium

Circulation Research Compendium on Sudden Cardiac Death

The Spectrum of Epidemiology Underlying Sudden Cardiac Death

Sudden Cardiac Death Risk Stratification

Genetics of Sudden Cardiac Death

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Gordon Tomaselli, Editor

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. Because conventional antiarrhythmic 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. (*Circ Res.* 2015;116:1937-1955. DOI: 10.1161/CIRCRESAHA.116.304691.)

Key Words: cardiac arrhythmias ■ death, sudden, cardiac ■ metabolism ■ oxidative stress

Sudden cardiac death (SCD) refers to death after 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 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 causal disorders for SCD in adults

aged ≥ 35 are coronary heart disease (65%–80%)^{2,3} and dilated cardiomyopathy (10%–15%).³ Various types of cardiomyopathy (eg, hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, infiltrative, inflammatory, and valvular diseases), genetically determined rhythm disorders (eg, 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

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Nonstandard Abbreviations and Acronyms

$\Delta\Psi_m$	mitochondrial membrane potential
AMPK	AMP-activated protein kinase
APD	action potential duration
CaMKII	calcium/calmodulin-dependent protein kinase II
Cav1	caveolin-1
DAD	delayed afterdepolarization
EAD	early afterdepolarization
ETC	electron-transport chain
FADH₂	flavin adenine dinucleotide
I_{CaL}	L-type Ca ²⁺ currents
I_K	delayed rectifier K ⁺ currents
IMAC	inner membrane anion channel
I_{Na}	Na ⁺ -current
IPC	ischemic preconditioning
I_{to}	transient outward K ⁺ current
Kv	voltage-gated K ⁺ channels
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
OXPHOS	oxidative phosphorylation
O_2^{*-}	superoxide
PK	protein kinase
PTP	mitochondrial permeability transition pore
RAS	renin–angiotensin system
ROS	reactive oxygen species
RyR2	ryanodine receptor 2
sarcoK_{ATP}	sarcolemmal K _{ATP}
SCD	sudden cardiac death
SERCA	sarco/endoplasmic reticulum Ca ²⁺ -ATPase
SR	sarcoplasmic reticulum

and causes of SCD have been extensively reviewed previously⁴ and in the articles on epidemiology of sudden cardiac death in this compendium series.

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 and pulseless ventricular tachycardia, are the most common electrophysiological mechanisms leading to SCD.^{5,7} Other physiological events that can result in SCD include pulseless electric activity, bradyarrhythmias, and asystole.^{8,9} This review will focus only on SCDs attributed to ventricular tachyarrhythmias.

Ventricular tachyarrhythmias, like all cardiac arrhythmias, result from 1 of 3 primary mechanisms: reentry, abnormal automaticity, or triggered activity. Reentry is the most common mechanism for ventricular tachyarrhythmias, involving the presence of an anatomic or functional disturbance of cardiac electric 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 3 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 noncardiac 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 coronary heart disease.^{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 mellitus 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 electric activity involving the initiation of the electric impulse from pacemaker cells, the propagation of the electric 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 electric impulse depends on low resistance pathways between cells via gap junctions, which are formed by docking of 2 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 Ca²⁺ 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 Ca²⁺ influx during the

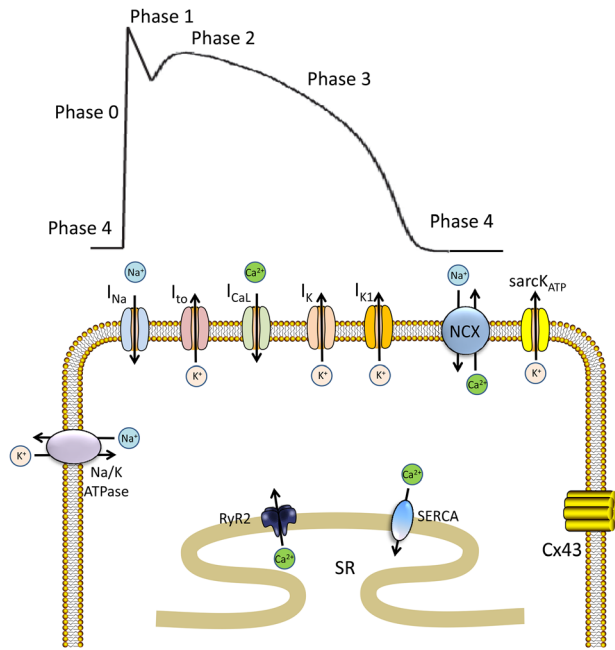


Figure 1. Action potential (AP) waveform and major ion channels/transporters in human ventricular cardiac myocytes. The AP waveform begins with the activation of Na^+ channels (I_{Na}), leading to the rapid depolarization (phase 0), followed by inward repolarizing transient outward K^+ currents (I_{to}) 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, SR/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) retrieves cytosolic Ca^{2+} to SR, and $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) extrudes Ca^{2+} from the cell, bringing cytosolic $[\text{Ca}^{2+}]$ back to baseline. Sarcolemmal K^+ ATPase channels (sarcK_{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. Cx43 indicates connexin 43.

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 $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX), an antiporter membrane protein extruding 1 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 electric functioning, leading to ventricular arrhythmias and SCD.

Myocardial Metabolism and Energetics

Under physiological conditions, >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 cycle.²² As the predominant energy generator in the heart, mitochondria occupy $\approx 30\%$ 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 used to generate the reducing equivalents, nicotinamide adenine dinucleotide (NADH), and flavin adenine dinucleotide (FADH_2), in the tricarboxylic acid cycle. NADH and FADH_2 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 O_2 to form H_2O . 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 ($\Delta\Psi_{\text{m}} = -150$ 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% to 90% of acetyl-CoA comes from β -oxidation of fatty acids, whereas 10% to 40% comes from oxidation of pyruvate derived from glycolysis and lactate oxidation.^{25,26} Although the majority of cardiac ATP is consumed by the myofilaments, it is estimated that $\approx 25\%$ 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 use 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 (O_2) 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 ($\text{O}_2^{\cdot-}$) and hydroxyl radicals, or in nonradical forms, such as hydrogen peroxide (H_2O_2), hypochlorite, nitric oxide (NO^{\cdot}), and peroxynitrite. 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 oxidation potential.

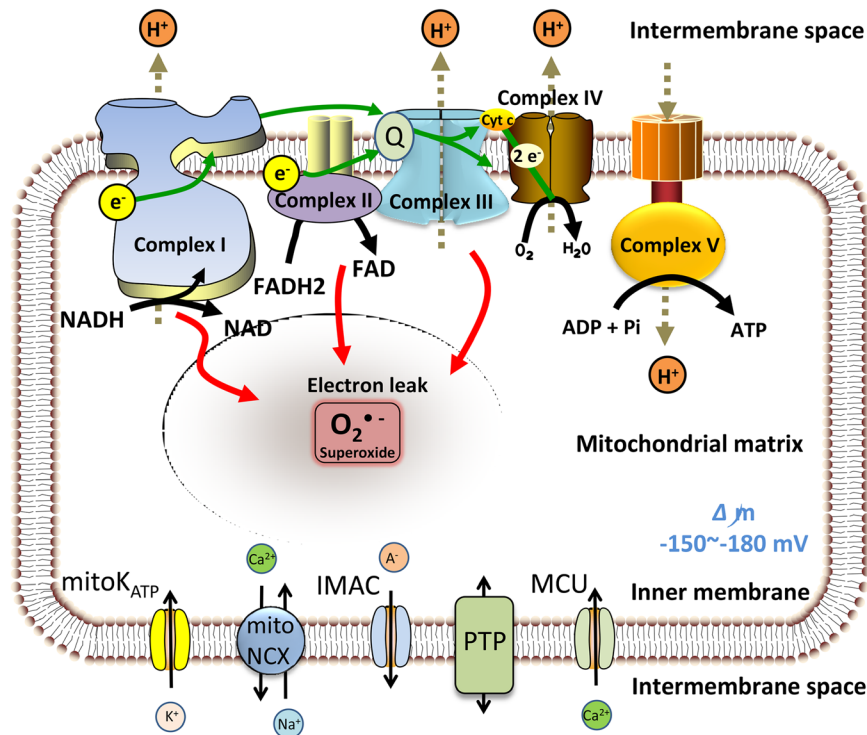


Figure 2. Oxidative phosphorylation and reactive oxygen species (ROS) production in mitochondria. The diagrams of the mitochondrial inner membrane show key components of the electron transport chain (ETC) above, and channels and transporters (below). Reducing equivalents nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH₂) produced from the tricarboxylic acid (TCA) cycle feed electrons to the ETC along the mitochondrial inner membrane. The electrons flow through the ETC with the following sequence: complex I and 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 ($\Delta\Psi_m$ = -150 to -180 mV). The free energy stored in the proton gradient and $\Delta\Psi_m$ then drive H⁺ through the mitochondrial ATP synthase (complex V), converting ADP to ATP. An estimated 0.1% to 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 (mito-K_{ATP}), the mitochondrial Na⁺/Ca²⁺ exchanger (mito-NCX), 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.

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 nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 2-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 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 O₂^{•-} from O₂.⁴⁰ Xanthine oxidase catalyzes the oxidation of hypoxanthine/xanthine and produces O₂^{•-}.⁴¹ NOS, on the contrary, produces a single nitrogen radical (NO[•]) through metabolizing L-arginine to L-citrulline. NOS becomes uncoupled under conditions such as depletion of L-arginine, oxidation of the NOS cofactor tetrahydrobiopterin, or increased S-glutathionylation of NOS. Under these conditions, NOS produces O₂^{•-} instead of NO[•].^{42,43} NO[•] 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 or S-glutathiolation (GSS-);^{44,45} NO[•] also reacts with O₂^{•-} and forms a more potent oxidative molecule peroxynitrite. Oxidative molecules derived from NO[•], such as NO₂[•], N₂O₃, and peroxynitrite, are termed reactive nitrogen species.^{44,45} As the metabolic hub in cardiac myocytes, mitochondria consume most of the O₂ 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% to 1% of the electrons passing through the ETC prematurely leak to O₂ at complexes I, II, or III,

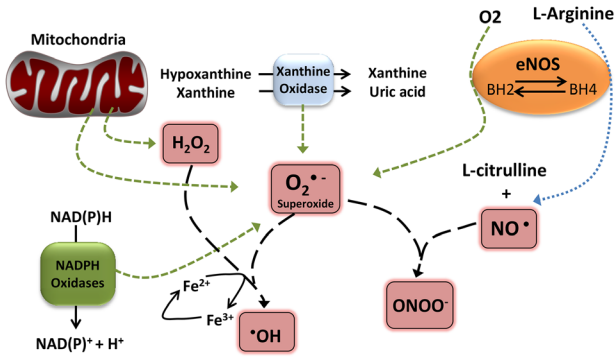


Figure 3. Major reactive oxygen species (ROS) generation pathways in cardiac myocytes. Mitochondria represent the predominant source of ROS in cardiac myocytes, producing superoxide ($O_2^{\bullet-}$) by the premature leak of electrons from the respiratory chain at complexes I, II, or III. Mitochondrial manganese superoxide dismutase can dismutate $O_2^{\bullet-}$ to hydrogen peroxide (H_2O_2), which can be broken down by transition metals such as Fe^{2+} and converted to hydroxyl radicals ($\bullet OH$). Superoxide can also be generated by the reduction of O_2 by free electrons released from (1) NAD(P)H oxidases, which oxidize nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (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 NO^{\bullet}) to an uncoupled oxide (producing $O_2^{\bullet-}$) 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 NO^{\bullet} and generate peroxynitrite $ONOO^-$.

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 to coenzyme Q10, or when the O_2 concentration is raised above physiological levels.^{47,48} Paradoxically, mitochondrial ROS production also increases under condition of low local O_2 concentration.^{47,49} Mitochondrial $O_2^{\bullet-}$ is converted to H_2O_2 and O_2 by manganese superoxide dismutase, 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 also at the same time optimizes ROS elimination through NADPH-dependent ROS scavenging system, such as glutathione 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 oxidized or 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}

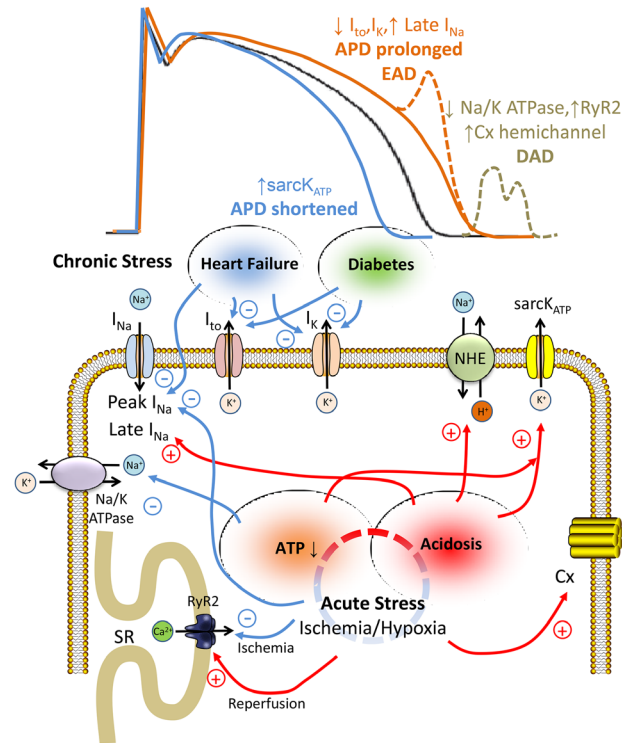


Figure 4. Effects of metabolic stresses on cardiac ion channel/transporter function. Schematic illustration of the impact of acute (ischemia/hypoxia) and chronic (heart failure and diabetes mellitus) metabolic stresses on cardiac ion channel/transporter function and the electrophysiological consequences. Acidosis during acute ischemia leads to opening of $sarK_{ATP}$ channels, increased activity of Na^+/H^+ exchanger (NHE), and increased late Na^+ -current (I_{Na}). ATP depletion during ischemia contributes to the opening of $sarK_{ATP}$ 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 (Cx) conduction, and reduced ryanodine receptor 2 (RyR2) activity. Reduced RyR2 activity during acute ischemia is rapidly reversed on reperfusion, contributing to the spontaneous waves of Ca^{2+} release and delayed afterdepolarizations (DADs). A few examples of electrophysiological impact from chronic metabolic stresses associated with heart failure and diabetes mellitus are also illustrated: heart failure reduces peak I_{Na} and multiple K_v (I_{to} , I_K) currents. Diabetes mellitus is also associated with reduction in K_v currents. The increase in $sarK_{ATP}$ currents lead to action potential duration (APD) shortening, whereas reduced K_v currents and increased late I_{Na} lead to prolonged APD and early afterdepolarizations (EADs). Finally, reduced Na/K ATPase activity, increased RyR2 activity, and increased gap junction conduction during ischemia predispose to arrhythmic DADs.

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 $\Delta\Psi_m$ and further acceleration of ROS production, a phenomenon termed mitochondrial ROS-induced ROS release.^{60–62} The PTP is a nonselective channel residing on the inner mitochondrial membrane, and it opens with elevated matrix $[Ca^{2+}]$, increased ROS and phosphate levels, and 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 contrary, is responsible for anionic currents across the mitochondrial membrane, allowing the passage of $\text{O}_2^{\cdot-}$ generated by ETC from the matrix to the cytoplasmic side of the inner membrane, thereby serving as a mitochondrial $\text{O}_2^{\cdot-}$ efflux and $\Delta\Psi_m$ depolarization mechanism.⁶⁹ During ischemia and reperfusion, mitochondrial $\text{O}_2^{\cdot-}$ production is increased, which leads to increased permeability of IMAC, resulting in a burst in ROS release and $\Delta\Psi_m$ depolarization. The resulting reversible $\Delta\Psi_m$ 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 $\Delta\Psi_m$ 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 IV,⁷¹ and ATP synthase,⁷⁰ impairing ATP generation and further aggravate electron leakage. In addition, $\text{O}_2^{\cdot-}$ 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 electric 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 cause and clinical course, can be divided into 2 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 electric remodeling.⁷⁴ Electric 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

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), PKA, and PKC, are involved in the regulation of these Ca^{2+} handling proteins (see review by Bers⁷⁶). 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 ischemia²⁸; however, RyR2 inhibition seems 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 On reperfusion or reoxygenation, 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; 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 and extrude Ca^{2+} mainly through the mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchanger (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+}

Table 1. Effects of Metabolic Changes on Cardiac Ion Channel/Transporter Function and Arrhythmogenicity

Channel/Transporter Effects	Metabolic Changes	Effects on Electric/Ionic Homeostasis	Proarrhythmic 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 mellitus, 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, ↑ on reperfusion	SR Ca ²⁺ load ↑, spontaneous Ca ²⁺ waves ↑	Ca ²⁺ transient/action potential alternans

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

dynamics under physiological and pathological conditions.¹⁰³ Recently, Williams et al¹⁰² provide quantitative data, suggesting that mitochondria do not function as a significant buffer of cytosolic Ca²⁺ under physiological conditions, with prolonged elevation of cytosolic [Ca²⁺]; however, mitochondrial Ca²⁺ uptake can increase ≤1000-fold and affect cellular Ca²⁺ dynamics significantly.¹⁰² Nevertheless, it has been shown that mitochondrial dysfunction results in cardiac myocytes Ca²⁺ transient alternans by impairing the mitochondrial capacity of Ca²⁺ handling, thereby predisposing to cardiac arrhythmias.¹⁰⁴ In addition, pharmacological inhibition of mitochondrial uptake through mitochondrial Ca²⁺ uniporter 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 Ca²⁺, membrane excitability, and electric functioning.

Metabolic Regulation of Na⁺ Homeostasis and Na⁺ Channel Function

Intracellular [Na⁺] plays a critical role in regulating the energetics, electric functioning, and contractility of cardiac myocytes, which can be attributed to the direct and indirect effects of cytosolic [Na⁺] on Ca²⁺ homeostasis,⁹² mitochondrial function,¹⁰⁶ and cellular signaling.¹⁰⁷ Multiple ion channels/transporters function to maintain the [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 electric conduction velocity in the myocardium. There are 2 effects of Na⁺ channel/transporter dysfunction on

arrhythmogenesis: (1) through affecting Na⁺-Ca²⁺ homeostasis, thereby promoting delayed afterdepolarizations and creating reentry substrates and (2) through electrophysiological effects of the channel function. For example, increased late I_{Na} predisposes to action potential duration (APD) prolongation and EADs, both of which are arrhythmogenic and predispose to SCD (Table 1; 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 [Na⁺], thereby leading to Ca²⁺ 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 hemichannels.^{112,113} Under normal conditions, connexin hemichannels located on adjacent intercellular sarcolemmal membranes combine to form gap junctions at the intercalated discs between cardiac myocytes. During ischemia/metabolic inhibition, connexin hemichannels localize to the nonjunctional membrane, turning into nonselective cation channels that are permeable to Na⁺, K⁺, and Ca²⁺.^{112,114} With the high conductance of these connexin hemichannels, it is suggested that the opening of even a small number of connexin 43 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; Figure 4).

Elevated cytosolic [Na⁺] also impairs cardiac metabolism reciprocally through affecting mitochondrial function. Mitochondrial [Na⁺] is regulated by NHE-mediated Na⁺ uptake¹¹⁶ and mitochondrial Na⁺-Ca²⁺ exchanger-mediated Na⁺ efflux.¹¹⁷ Under physiological conditions, energized mitochondria extrude H⁺, and the resulting pH gradient drives the Na⁺ gradient between mitochondrial matrix (lower [Na⁺]) and cytosol (higher [Na⁺]).^{116,118} Intracellular Na⁺ levels increase significantly in pathological conditions, such as myocardial

ischemia.¹⁰⁹ The rise in cytosolic $[Na^+]$ during ischemia widens the Na^+ gradient across mitochondria, leading to stronger driving force for Ca^{2+} extrusion from mitochondria via mitochondrial Na^+-Ca^{2+} exchanger, thereby leading to reduced mitochondrial $[Ca^{2+}]$ and altered mitochondrial energetics,^{100,106} including decreased activities of Ca^{2+} -dependent dehydrogenases in the tricarboxylic acid 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 $[Na^+]$, impaired mitochondrial metabolism, increased ROS emission, and ROS-induced elevation of cytosolic $[Na^+]$.¹²⁵

In addition to cytosolic $[Na^+]$, cardiac metabolism also influences electric 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, for example, has been shown to decrease peak Na^+ current (peak I_{Na}) and to slow its inactivation.¹²⁷ In addition, lysophosphatidylcholine 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; Figure 4).^{89,90} Cardiac Na^+ channels are reported to be modulated by AMP-activated PK (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 electric 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

Metabolic Regulation of K^+ Channels and Cardiac Repolarization

Various types of voltage-gated K^+ (K_v) channels and nonvoltage-gated inwardly rectifying channels contribute to myocardial action potential repolarization.^{19,20} Under pathological conditions, such as myocardial ischemia, diabetes mellitus, and heart failure, repolarizing K_v 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 inwardly rectifying K^+ 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} ($sarK_{ATP}$) channels, 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 mellitus, one of the leading chronic metabolic disorders associated with ventricular arrhythmias and SCD.^{138,139} Studies on cellular mechanisms of diabetes mellitus-induced repolarization abnormalities have consistently revealed downregulation of cardiac K_v currents in diabetic heart.^{140–142} Multiple mechanisms have been proposed to account for reduced K_v currents during diabetes mellitus. First, post-translational inhibition of K_v channel function occurs in diabetes mellitus. Plasma levels of free fatty acid metabolites, such as palmitoylcarnitine and palmitoyl-CoA, are increased with diabetes mellitus. They have been shown to inhibit cardiac K_v currents directly.¹⁴³ Acute treatment with insulin has been demonstrated to reverse diabetes mellitus-induced K_v current reduction, suggesting the primary role of insulin-dependent signaling on K_v channel function.¹⁴⁰ It has been suggested that improved glucose utilization by insulin may be responsible for restored K_v current expression because compounds known to improve glucose utilization (such as dichloroacetate or L-carnitine) have been shown to normalize cardiac K_v current density with diabetes mellitus.¹⁴¹ Increased ROS levels also contribute to reduced cardiac K_v currents during diabetes mellitus, and direct incubation with antioxidant glutathione has been shown to restore K_v current expression in myocytes isolated from diabetic hearts.¹⁴⁰ Second, transcriptional regulation of K_v channel expression may reduce K_v currents. Peroxisome proliferator-activated receptor α , a critical regulator of glucose/fatty acid metabolism, is upregulated in diabetic heart and has been suggested to transcriptionally repress cardiac K_v channel expression.¹⁴⁴ Cardiac-specific overexpression of peroxisome proliferator-activated receptor α downregulates the mRNA and protein expression levels of $K_v4.2$ and $KChIP2$, the channel subunits encoding I_{to} ,¹⁴⁴ whereas peroxisome proliferator-activated receptor α knockout upregulates I_{to} .¹⁴⁴ Interestingly, it has been recently demonstrated that the activation of phosphoinositide 3-kinase α , a key component of insulin receptor signaling pathway that can be triggered by insulin treatment or exercise training, upregulates mRNA levels of various K_v channels^{145,146} through an Akt-independent mechanism,¹⁴⁷ which may provide the mechanistic explanation for insulin-mediated K_v current modulation, as well as the metabolic impact of exercise training on cardiac repolarization. It is important to note that the changes in K_v current observed with diabetes mellitus or exercise training reflect the chronic effects of metabolic stress on K_v channel remodeling, rather than acute channel effects.

The $sarK_{ATP}$ channels are present at high density in the sarcolemmal membrane. $sarK_{ATP}$ 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 On increased metabolic stress with ATP depletion and acidosis (eg, during myocardial ischemia),¹⁴⁹ $sarK_{ATP}$ channels are activated, allowing an inwardly rectifying repolarizing K^+ current. $sarK_{ATP}$ 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 sarK_{ATP} channels closed) until 10 to 15 minutes after the onset of ischemia.¹⁵¹ Therefore, ATP depletion may not contribute to sarK_{ATP} channel opening during the early phase of ischemia.

The cell surface expression density of sarK_{ATP} channels is high. It has been estimated that the opening of merely 1% of the sarK_{ATP} channels is sufficient to significantly shorten myocardial APD.¹⁵² The opening of sarK_{ATP} channels shortens APD and decreases inward Ca^{2+} currents, thereby reducing Ca^{2+} -mediated cardiac energy consumption and preventing Ca^{2+} overload-induced cell death. With adequate numbers of sarK_{ATP} channels in the open state, however, cardiac myocytes become hyperpolarized and rendered unexcitable.¹⁵³ This creates a current sink that slows or blocks electric propagation in the myocardium, predisposing to the development of ventricular arrhythmia (Table 1; Figure 4).^{62,154} Indeed, pharmacological inhibition of sarK_{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 the activation of sarK_{ATP} channels. During ROS-induced ROS release, for example, dissipation of $\Delta\Psi_{\text{m}}$ results in cessation of mitochondrial ATP production, leading to sarK_{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 sarK_{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 sarK_{ATP} channel macrocomplex and regulate its function.^{163,164} sarK_{ATP} channels have also been shown to exhibit reduced sensitivity to ATP-mediated inhibition in diabetic heart, resulting in higher sarK_{ATP} conductance and greater APD shortening in response to hypoxia.¹⁶⁵

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 $\gamma 2$ 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 lysophosphatidylcholine, 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 electric 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

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 contrary, 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; 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 contrary, 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 non-ischemic 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

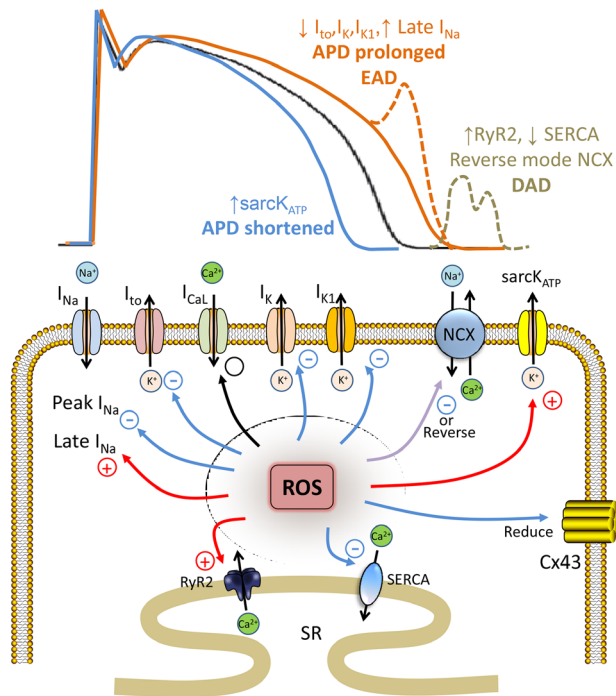


Figure 5. Effects of increased oxidative stress on cardiac ion channel/transporter function. Schematic figure to demonstrate the impact of increased oxidative stress on cardiac ion channel/transporter function. Increased reactive oxygen species (ROS) enhance the activity of ryanodine receptor 2 (RyR2) and sarcoK_{ATP} channels and increase late I_{Na}. Excessive oxidative stress reduces peak I_{Na} and repolarizing K⁺ currents (transient outward K⁺ current [I_{to}], delayed rectifier K⁺ currents [I_K], and I_{K1}) and leads to reduced gap junction protein connexin (Cx) 43 and reduced sarco/endoplasmic reticulum Ca²⁺-ATPase (SERCA) activity. Increased ROS inhibit normal operation of Na⁺/Ca²⁺ exchanger (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 action potential duration (APD) shortening, whereas reduced Kv currents and increased late I_{Na} lead to prolonged APD and early afterdepolarizations (EADs). Increased RyR2 activity, reduced SERCA function, and reverse mode NCX activity lead to cytosolic Ca²⁺ overload and predispose to delayed afterdepolarizations (DADs).

altering membrane Na⁺ channel protein expression levels.⁵⁷ The reduced peak I_{Na} can be restored by mitochondrial antioxidants NAD⁺ or Mito-TEMPO [(2,2,6,6-Tetramethylpiperidin-1-yl) oxyl] 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 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 glycerol-3-phosphate dehydrogenase 1-like 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 Myocardial ROS and Potassium Channels Section of this article), 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 glutathione 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

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 low-density lipoprotein has been demonstrated to stimulate I_{CaL} through lysophosphatidylcholine-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 on 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 Ca²⁺ from cytosol in exchange for Na⁺, has been shown to be activated in the reverse mode by increased oxidative stress, thereby increasing cytosolic [Ca²⁺] (Table 2; 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 Ca²⁺ leak, promoting cytosolic Ca²⁺ overload and delayed afterdepolarizations.²⁰⁰ Taken together, the net impact of increased oxidative stress

Table 2. Effects of Increased Oxidative Stress on Cardiac Ion Channel/Transporter Function and Arrhythmogenicity

Channel/Transporter	Effects of ROS	Effects on Electric/Ionic Homeostasis	Proarrhythmic 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
Mito-NCX	Reverse mode	Ca^{2+} overload	DAD
Mito- K_{ATP}	↑	↑ Mitochondrial K^+ influx	Protective, ischemic-preconditioning

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

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 delayed afterdepolarizations 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 $[Ca^{2+}]$ by altering mitochondrial ion channel activities, contributing to the perturbation of cytosolic Ca^{2+} homeostasis. For example, increased ROS during myocardial ischemia depolarize $\Delta\Psi_m$,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 $[Ca^{2+}]$ increase, leading to increased ROS production. ROS overproduction induced by elevated mitochondrial $[Ca^{2+}]$ 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 electric 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 mitochondrial Na^+-Ca^{2+} exchanger),^{119,120} which

also favors ROS production (by impairing mitochondrial energetics and oxidizing NAD(P)H).^{119,120}

NO^* and peroxynitrite also play important roles in modulating cardiac Ca^{2+} channel and Ca^{2+} -handling proteins. Increased NO^* 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 NO^* on RyR2 is concentration dependent. Physiological NO^* levels ($\approx 1 \mu\text{mol/L}$) do not affect RyR2 activity, whereas supraphysiological levels ($\geq 100 \mu\text{mol/L}$) inhibit RyR2 activity.²¹⁴ Peroxynitrite, on the contrary, 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 phospholamban from SERCA2a.²¹⁵

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 glutathione 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 PKs 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

$\Delta\Psi_m$ and ATP production levels, which result in oscillation of sarcK_{ATP} currents and APDs.^{69,225} The $\Delta\Psi_m$ 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 $\Delta\Psi_m$ depolarization on increased ROS; pharmacological inhibition of IMAC has been shown to prevent $\Delta\Psi_m$ depolarization and the oscillation of sarcK_{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 $\Delta\Psi_m$, which leads to a compensatory increase in proton pumping and cellular respiration to maintain $\Delta\Psi_m$ and ETC activity.²²⁹ Partially dissipated $\Delta\Psi_m$ induced by mito-K_{ATP} channel opening blunts mitochondrial Ca²⁺ accumulation during ischemia and reduces PTP opening on 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 postischemic 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 Pharmacological inhibition of mito-K_{ATP} channels abrogates the antiarrhythmic effects of IPC,²³⁸ and several mito-K_{ATP} channel openers are shown to provide protective effects against ischemia-induced cardiac arrhythmias.^{157,239,240}

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 6 connexin (connexin) proteins. Gap junctions mediate the intercellular communication of small metabolites and ions and play an essential role in cardiac electric conduction.²⁴¹ Among the 3 principal connexin isoforms (connexin 40, connexin 43, and connexin 45) expressed in the heart, connexin 43 is the predominant isoform expressed in ventricular myocytes.²⁴² Ventricular connexin 43 expression has been shown to be downregulated with myocardial ischemia,^{243,244} and heart failure,^{245,246} which can lead to slowed conduction, increased electric 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 on 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 connexin 43.^{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 connexin 43 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 connexin 43 downregulation, impaired gap junction function, slowed cardiac conduction, and increased incidence of ventricular arrhythmias and SCD.^{35,257} The downregulation of connexin 43 and increased risk for arrhythmias in angiotensin-converting enzyme 8/8 mice are alleviated by pharmacological inhibition of RAS²⁵⁸ and cSrc.²⁵⁷ It has been shown that increased myocardial p-cSrc leads to connexin 43 reduction through the competition between p-cSrc and connexin 43 for the binding with zonula occludens-1, a scaffolding protein at the intercalated disk, resulting in connexin 43 destabilization and degradation.²⁵⁹ Elevated p-cSrc levels also impair gap junction function by phosphorylating connexin 43 at tyrosine residues.²⁶⁰ Using the same ACE 8/8 mouse model, we have also shown that cardiac mitochondrial ROS were markedly increased with enhanced RAS activity.^{35,257} Treatment with mitochondria-targeted antioxidant Mito-TEMPO, but not with other types of antioxidants, decreases cSrc phosphorylation, preserves connexin 43 expression, improves gap junction function, and abolishes ventricular arrhythmias and SCD in ACE 8/8 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 of cardiac caveolin-1 (Cav1), an intrinsic inhibitor of cSrc, resulting in Cav1-cSrc dissociation and subsequent cSrc activation. Cav1 S-nitrosylation upon enhanced RAS signaling is mediated by increased Cav1-eNOS binding that is dependent on elevated mitochondrial ROS.²⁶² Consistent with these findings, Cav1 knockout mice exhibit increased cSrc activation, reduced connexin 43 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 connexin 43 and increased risk for ventricular arrhythmias and SCD, it would be of great interest to test whether the treatment with mitochondria-targeted antioxidant

Table 3. Potential Novel Therapeutics Against Ventricular Arrhythmias and Sudden Cardiac Deaths 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 $\Delta\Psi_m$ depolarization	Prevent mitochondrial ROS-induced ROS release and subsequent Ca ²⁺ overload
IMAC inhibitor ^{62,227,228}	Prevent ROS-induced $\Delta\Psi_m$ depolarization	Prevent mitochondrial ROS-induced ROS release and subsequent Ca ²⁺ overload
Mito-K _{ATP} opener ^{229,230}	\uparrow Mitochondrial K ⁺ influx, prevents $\Delta\Psi_m$ depolarization	Stabilize $\Delta\Psi_m$ and maintain mitochondrial function
Mitochondria-targeted antioxidants ^{35,57,262}	\downarrow Mitochondrial ROS	Alleviate Cx43 and peak I _{Na} downregulation with increased mitochondrial ROS
CaMKII δ inhibitor ¹²⁴	Prevent ROS-induced late I _{Na}	Prevent ROS-induced late I _{Na} and EAD

$\Delta\Psi_m$ indicates mitochondrial membrane potential; CaMKII, calcium/calmodulin-dependent protein kinase II; Cx 43, connexin 43; DM, diabetes mellitus; EAD, early afterdepolarization; HF, heart failure; IMAC, mitochondrial inner membrane anion channel; I_{Na}, Na⁺-currents; MCU, mitochondrial calcium uniporter; NHE, Na⁺-H⁺ exchanger; PI3K α , phosphoinositide 3-kinase α ; and PTP, mitochondrial permeability transition pore; and ROS, reactive oxygen species.

can normalize connexin 43 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 disease,²⁶⁵ and diabetes mellitus,^{138,139} also result in arrhythmogenic changes that predispose to SCDs. Many of the arrhythmogenic changes related to these chronic conditions involve electric 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 electric 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 K_v 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. Additional studies are required to address these possibilities.

Conclusions

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 electric 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. Because the conventional antiarrhythmic drugs targeting ion channels are often proarrhythmic, 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.

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