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Metabolic Stress, Reactive Oxygen Species, and Arrhythmia

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Abstract

Cardiac arrhythmias can cause sudden cardiac death (SCD) and add to the current heart failure (HF) health crisis. Nevertheless, the pathological processes underlying arrhythmias are unclear. Arrhythmic conditions are associated with systemic and cardiac oxidative stress caused by reactive oxygen species (ROS). In excitable cardiac cells, ROS regulate both cellular metabolism and ion homeostasis. Increasing evidence suggests that elevated cellular ROS can cause alterations of the cardiac sodium channel ($Na_v1.5$), abnormal Ca^{2+} handling, changes of mitochondrial function, and gap junction remodeling, leading to arrhythmogenesis. This review summarizes our knowledge of the mechanisms by which ROS may cause arrhythmias and discusses potential therapeutic strategies to prevent arrhythmias by targeting ROS and its consequences.

Keywords

reactive oxygen species; sodium channel; Ca^{2+} handling; mitochondria; connexin; arrhythmia

1. Introduction

The majority of sudden cardiac death (SCD) results from the occurrence of potentially lethal ventricular tachycardia (VT) or ventricular fibrillation (VF), only two of many types of arrhythmia. Arrhythmia is an irregular heart rhythm and is classified by rate as either tachycardia or bradycardia (resting heart rate >100 beats/min or < 60 beats/min, respectively). Arrhythmias are also mechanistically classified as automatic, reentrant, and triggered. Reentry is favored by slow, inhomogeneous conduction. Types of arrhythmia include (1) premature beats; (2) supraventricular arrhythmias (e.g., atrial fibrillation (AF),

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Disclosures

SCD has patents pending on 1) Mitochondrial anti-oxidants for prevention of sudden death, 2) a method for modulating or controlling sodium channel current by ROS originating from mitochondria, 3) Activation of the renin-angiotensin system and SCD, 4) Oxidative stress markers predict AF, and 5) Modulation of sodium channels by NAD. Dudley SC is sole owner of ROS Technologies, Inc., a medical diagnostics company developing a blood test to predict sudden death risk in patients with heart failure.

atrial flutter, and paroxysmal supraventricular tachycardia); (3) ventricular arrhythmias (e.g., VT and VF); and (4) bradyarrhythmias.

SCD occurs in approximately 180,000 – 250,000 cases annually in the United States, and an estimated 4-5 million cases worldwide [1]. SCD occurs in hypertrophic cardiomyopathy, dilated cardiomyopathies, arrhythmogenic right ventricular dysplasia, myocardial infiltrative diseases, and other related disease states [2]. The prevalence of cardiovascular diseases potentially associated with lethal ventricular arrhythmia is estimated at approximately 13 million US individuals, which is about 5% of the middle-aged population [3].

Paroxysmal or persistent AF afflicts approximately 2.2 million Americans in addition to 4.5 million people in the European Union. AF is a supraventricular tachyarrhythmia characterized by uncoordinated atrial activation with consequential deterioration of atrial mechanical function. It is the most common arrhythmia clinically encountered, accounting for over 30% of hospital admissions for cardiac rhythm disturbances [4] and is associated with increased risks for stroke, heart failure (HF), and death [5, 6]. The incidence of AF noticeably increases over the age of 60, afflicts 3-5 % of the population 65 to 75 years old and occurs in up to 8% of those older than 80 years [7-9]. The prevalence of this arrhythmia has significantly increased recently, and the number of Americans with AF is expected to surpass 5 million by 2050 [10].

Despite the high prevalence and significance of arrhythmias, the mechanisms of arrhythmogenesis are not fully understood. Some molecular mechanisms known to contribute to arrhythmias include genetic alterations of ion channels leading to electrophysiological dysregulations and structural remodeling of the left ventricle (LV) in hypertrophy and HF [11-14]. Increasing evidence suggests that altered cardiac ion homeostasis and structural remodeling are highly associated with elevated reactive oxygen species (ROS) and metabolic stress [15, 16]. In this review, we summarize possible mechanisms whereby the imbalanced cellular redox state may cause arrhythmogenesis by ROS-induced alterations of ion homeostasis and ion channel behavior.

2. Cardiac conditions associated with metabolic stress, ROS, and arrhythmias

Cardiac metabolism is reflected by adenosine-5′-triphosphate (ATP), which is the source of energy for maintenance of ion homeostasis as well as repetitive mechanical contraction and relaxation. Approximately 60-70% of ATP is used for cardiac muscle contraction, and the remaining 30-40% is used for Ca^{2+} uptake into the sarcoplasmic reticulum (SR) to initiate diastolic relaxation and to sustain ion current homeostasis including the maintenance of Na^{+} and K^{+} gradients across the plasma membrane [17, 18].

Mitochondria occupy approximately 30% of the volume of ventricular cardiomyocytes and form a network around the myofilaments resulting in the location of ATP production sites being adjacent to ATP consumption sites [19]. Although there are several sources of ROS in cardiac muscle including NADPH oxidase, xanthine oxidase, and uncoupled NOS, mitochondria are the major ROS source. Electron leakage from complex I and III is

associated with the generation of ROS in the failing heart [20], ischemia/reperfusion [21] and arrhythmia [16]. Complex I (i.e. NADH and ubiquinone oxidoreductase) produces superoxide ($O_2 \cdot^-$) in the mitochondrial matrix whereas complex III (i.e. Q-cycle, cytochrome bc1 complex and coenzyme Q) produces superoxide in the matrix and intermembrane space [22]. Oxidative phosphorylation is tightly linked to mitochondrial regulation so that the cellular ATP content remains constant even with large increases in cardiac ATP consumption [23]. The electron transport chain (ETC) of the mitochondria matrix transfers electrons from carbon substrates (e.g., fatty acids and pyruvate) to nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) in order to synthesize ATP. During the occurrence of abnormal pathophysiological conditions that cause arrhythmia (when the balances of blood flow, oxygen delivery, mitochondrial metabolism, NADH formation, and ATP synthesis are disrupted), NADH, protons, and lactate accumulate, potentially contribute to arrhythmic risk. Moreover, when disrupted, mitochondria produce ROS via electrons leaking from the ETC that react with molecular oxygen to form superoxide. The accumulation of ROS in the mitochondria can lead to oscillations in the mitochondrial membrane potential ($\Delta\Psi_m$) and changes in matrix concentrations of Ca^{2+}, NADH, ADP, and tricarboxylic acid (TCA) cycle intermediates [24]. Hypoxia also decreases the rate of metabolism, resulting in the decrease of intracellular $NADH/NAD^+$ ratio and an increase in ROS production [25-27]. Accumulating ROS further increases the rate of ROS production, a phenomenon known as ROS induced ROS release [28].

ROS, also called reactive oxygen intermediates, are comprised of superoxide, hydrogen peroxide (H_2O_2), hydroxyl radicals ($\cdot OH$) and peroxynitrite ($ONOO^-$) [29]. ROS are highly reactive and unstable molecules leading to irreversible, deleterious reactions with lipids and proteins. Cardiac conditions such as hypertension [30], diabetes mellitus [31], coronary artery disease [32, 33], cardiomyopathies, and HF [34, 35] are associated with altered metabolism and arrhythmias. In diabetes mellitus, arrhythmias and oxidative stress are well correlated [31]. In ischemia/reperfusion injury, the mitochondrial ETC serves as the major source of ROS [21]. In chronic HF, ROS levels increase [20, 36] and myocardial antioxidant reserve decreases [37, 38].

Trace amount of ROS serve as signaling molecules in physiological conditions, but the excessive production of ROS elicits pathologic cellular processes [39]. To protect cellular functions from ROS, cells have two defense mechanisms, enzymatic and nonenzymatic pathways. The enzymatic pathway includes three superoxide dismutases, catalase, and glutathione peroxidase [40]. The dismutation of superoxide by superoxide dismutase results in the generation of H_2O_2, which catalase further metabolizes to water and oxygen. In the nonenzymatic pathways, there are a variety of redox-defense system including antioxidant scavengers, such as vitamins E and C [41], the ratio of reduced glutathione (GSH) to oxidized glutathione disulfide (GSSH), the ratio of oxidized and reduced forms nicotinamide adenine dinucleotide, $NADH/NAD^+$, and the thioredoxin [14, 42]

One of the main consequences of oxidative stress is the depletion of key intracellular antioxidants such as glutathione (GSH), the largest antioxidant pool in the heart [43]. In ischemia [44-46], HF [47], and type 2 diabetes [48], conditions associated with arrhythmia,

the oxidized GSH ratio (GSH/GSSH) of ~200-300 to 1 is decreased by 50-70%. During AF, there is biochemical evidence of oxidation by peroxynitrite and hydroxyl radicals [49]. Systemically, serum oxidative markers are elevated in individuals with AF [50]. Other oxidative serum markers including malondialdehyde and nitrotyrosine are also increased in arrhythmias [20, 51].

Another important cellular redox defense system is a cytosolic NADH/NAD^+ level [43]. HF is associated with reduced NAD^+ and increased NADH [52-54]. The balance of oxidized and reduced NAD forms varies some between species and tissues. In the monkey, the estimated ratio between free NADH to NAD^+ in the cytoplasm is ~0.002. In rodents and swine, the ratio of total NADH/NAD^+ is ranges from 0.05 to 4, under normal aerobic conditions [27, 43, 55, 56]. These numbers are in the range of the cytosolic NAD redox potential of −250 mV, which implies a NAD^+/NADH ratio of ~500:1 [57]. In the mitochondrion, the concentration of NAD^+ is similar to that in the cytosol [58]. More than 80% of mitochondrial NADH is bound to proteins, so the free concentration is much lower [59] and the ratio of oxidized to reduced forms may be different in the mitochondria. In our previous work, an increase in cellular NADH by 1.7 fold increased mitochondrial ROS production by 2 fold [60]. This is change in the NAD^+/NADH ratio is on the order of that seen in heart failure [61, 62]. O'Rourke et al. have demonstrated repetitive and self-sustaining oscillations in NADH during mitochondrial injury [63]. Moreover, these changes in pyridine nucleotide levels are associated with ROS, and ROS may explain some arrhythmic effects. ROS bursts with NAD(P)H oxidation in single mitochondria [64], and isolated cardiac myocytes from guinea pigs [65]. Therefore, the NADH/NAD^+ ratio, a measure of both redox balance and metabolic activity, can directly affect ROS production and arrhythmic risk as discussed below. Moreover, O'Rourke and colleagues have posited that oxidative stress can occur at either extreme of redox potential, either highly reduced or highly oxidized [66]. They point out that ROS overflow can increase because of ROS production, as the redox environment becomes more reduced, or at oxidized redox potentials as a result of scavenger pool depletion.

Although there are many reports suggesting that oxidative stress is related to the pathogenesis of arrhythmias, the major sources of ROS and their regulating mechanisms are not fully understood. ROS can be generated through electron leakage from mitochondria during oxidative phosphorylation and through the activation of several cellular enzymes, including the NADPH oxidase, xanthine oxidase, and uncoupled nitric oxide synthase (NOS) (Fig. 1) [29, 39, 67, 68]. The NADPH oxidase complex is a significant cardiac ROS source [69]. In a swine rapid atrial pacing model of AF, increased activity of the NADPH oxidase and xanthine oxidase are associated with the production of superoxide and reduced nitric oxide (NO) bioavailability in both of the left atria (LA) and left atrial appendage (LAA) [70, 71]. Apocynin, a NADPH oxidase inhibitor, reduced LAA superoxide production by 91%, supporting the activation of NADPH oxidase in AF. Active Rac1, a small G-protein associated with NADPH oxidase activation, is increased by 6.9 fold in LAA of the AF model [70, 71]. Rapid atrial pacing has been shown to elevate myocardial peroxynitrite formation and leads to a shortening of the atrial effective refractory period, which can be reversed by treatment with ascorbate and statins. These drugs are known to decrease NADPH oxidase activation [72, 73]. Increased NADPH oxidase activity has been

observed in pressure-overload LV hypertrophy, myocardial infarction, HF, and AF [74-78]. Moreover, NADPH oxidase activation and NOS uncoupling could be important factors in the initiation of mitochondrial ROS generation. Rotenone, a mitochondrial complex I inhibitor, lowers the basal levels of superoxide detected in atria in patients of AF [79].

3. Mechanisms whereby ROS can affect arrhythmias

Arrhythmogenesis and oxidative stress are correlated, but how do ROS cause arrhythmia? Possibilities include Na^+, Ca^{2+}, and K^+ ion homeostasis changes and altered voltage-gated ion channel activity.

A. Regulation of $Na_v1.5$ by ROS

$Na_v1.5$ cardiac Na^+ channel encoded by the SCN5A gene is one of the key ion channels for excitability and rapid impulse propagation. An appropriate number of functional $Na_v1.5$ channels is critical for normal cardiac function [80]. Either excess or reduced channel current results in increased arrhythmic risk, as demonstrated in the inherited sudden death syndromes Long QT type 3 [81] and Brugada syndromes [82].

Potentially pro-arrhythmic transcription factors such as, peroxisome proliferator-activated receptor, c-fos and NF-κB, are known to be regulated by redox signalling pathways [83-85]. Among these transcriptional factors, NF-κB is one of key transcriptional regulator that mediates alterations of genes during biological states of stress such as injury, inflammation [78], hypertrophy [86], renin-angiotensin system (RAS) activation [87, 88], and oxidative stress [89]. Recently, it was shown that the promoter region of the cardiac sodium channel gene (SCN5A) contains a NF-κB binding sequence, suggesting that SCN5A is a gene regulated by NF-κB [90]. Angiotensin II (AngII) or H_2O_2 treatment of cardiomyocytes results in increased NF-κB binding to SCN5A promoter with subsequent reduction in transcriptional activity [87]. Simultaneous overexpression of the p50 and p65 NF-κB subunits recapitulates the effects of AngII or oxidative stress. Furthermore, a mutation in the NF-κB consensus sequence prevented this transcriptional downregulation. The reduction in I_{Na} seen with AngII is similar to its effect on other cardiac ion channels, including the transient outward current K^+ channel α-subunit ($K_v4.3$) [91], the gap junction protein connexin (Cx) 43 [92], Cx40 [92, 93], and the Ca^{2+} current [93], which may be mediated by comparable mechanisms and may also contribute to enhanced arrhythmic risk in states of increased oxidative stress. Inspecting the promoter regions of other channel and subunit genes reveals that the T-type Ca^{2+} channel, the transient outward current, the ultra-rapid delayed rectifier, and Cx40 also have NF-κB consensus binding sequences [14]. All appear to undergo transcriptional regulation during arrhythmias, but experimental evidence currently exists only for SCN5A to be regulated by NF-κB [87].

Aside from the direct NF-κB regulation on ion channel promoters, SCN5A gene expression is also regulated by alternative mRNA splicing. In human HF, a condition linked with increased risk of arrhythmia, three C-terminal truncated splice variants are observed [80, 94]. These variants do not form functional channels and result in a reduction in I_{Na}. Stimuli for this alternative splicing included hypoxia and AngII, factors associated with ROS and

arrhythmic risk. This mechanism is mediated by RBM 25 and LUC7L3, SCN5A splicing factors which are regulated by HIFα [95, 96].

As discussed above arrhythmic conditions with mitochondrial dysfunction and an accumulation of NADH [25, 26]. NADH increases mitochondrial ROS [25, 26]. Glycerol-3-phosphate dehydrogenase 1-like protein (GPD1-L) is involved in NAD-dependent energy metabolism, and mutations in the gene are associated with Brugada and sudden infant death syndromes [60, 97, 98]. Mutation of GPD1-L results in elevated NADH and $Na_v1.5$ downregulation, suggesting a mechanism that links metabolism, ROS, and arrhythmic risk [16, 60, 97, 98]. Intracellular NADH level leads to activation of protein kinase C and subsequent increased mitochondrial ROS production [60]. The immediacy of the NADH effect on reducing I_{Na} and the lack of change in mRNA abundance suggests that this effect of NADH is post-transcriptional. Biological significance was implied when programmed electrical stimulation (PES)-induced VT was demonstrated after pyruvate/lactate buffer perfusion, a condition under which intracellular $[NADH]_i$ was increased 1.7-fold. On the other hand, the oxidize intracellular pyridine nucleotide, NAD^+, ameliorated the mitochondrial ROS production, reduced I_{Na}, and inducibility in a mouse model in which one allele of the cardiac $Na_v1.5$ channel was ablated ($SCN5A^{+/-}$) (Fig. 2) [99]. Nevertheless, the NAD^+ effect did not seem to occur by the same signaling mechanism as did the NADH effect, and the NAD^+ effect could be recapitulated by a protein kinase A (PKA) activator or prevented by a PKA inhibitor (Fig. 3). This is consistent with the known effect of external NAD^+ to activate PKA in human granulocytes [100] and osteoblastic cells [101] and of PKA activation to increase I_{Na} [102, 103]. Although these findings suggest that the balance of oxidized and reduced NAD(H) may be a critical mechanism in the regulation of I_{Na} in the metabolic state of myocytes, there are still not fully investigated that redox potential differences between intracellular and mitochondria NADH affect to mitochondrial ROS elevation directly or indirectly via signaling pathways.

Changes in the I_{Na} induced by NADH are consistent with alterations observed in other channels. Tipparaju *et al*. [104, 105] have reported that NAD(P)H to $NAD(P)^+$ ratio regulates K^+ currents, although the regulation mostly affects gating rather than peak current. Some transient receptor potential currents are increased by NAD^+ in a manner similar to that seen in our experiments [106]. A non-selective cation channel conductance is increased by NAD^+ [107].

B. Mitochondrial ROS and the metabolic sink theory

O'Rourke and colleagues suggests that the mitochondria have an important role in the development of arrhythmia through metabolic stress that causes ADP levels to rise and ATP to be depleted. This leads to opening of sarcoplasmic potassium-sensitive K_{ATP} ($sarcK_{ATP}$) channels and a decreased cardiac action potential (AP) duration, a reduced Ca^{2+} transient, and an increased K^+ conductance [19, 26, 63, 108]. If enough channels open, cells can be rendered inexcitable by clamping the membrane potential close to potassium's Nernst equilibrium potential, thus creating a current sink for propagating depolarization waves [109]. This phenomenon produces cellular regions of inexcitability that have been termed "metabolic sinks" [110, 111], and they occur when a threshold of $sarcK_{ATP}$ channels open

[109]. Because current sinks create regions of inhomogeneous excitability, the development of reentrant arrhythmias is favored [112-114].

Even without complete conduction block, the opening of these channels can significantly shorten the cardiac AP, creating heterogeneity suitable for reentrant arrhythmia [110, 111, 115]. The behavior of the mitochondria and oxidative stress has been linked to fluctuations in AP duration [26, 63]. During oxidative stress, increases in the sarcK_{ATP} current have been shown to fluctuate in phase with the $\Delta\Psi_m$ [26, 113], demonstrating that arrhythmia may be interconnected with mitochondrial function. In a study conducted by Aon *et al.*, increases of superoxide anions were induced by a laser flash that also caused oscillations in the $\Delta\Psi_m$ [26], which could be prevented by blocking the activity of mitochondrial inner membrane anion channels (IMAC). These observed collapses in the $\Delta\Psi_m$ may be caused by ROS-induced ROS release [116]. Further confirmation for the role of IMAC involved ROS release from mitochondria and subsequent arrhythmia was observed in a series of studies where the inhibition of IMAC prevented arrhythmia in intact mammalian hearts [110, 112, 117]. Furthermore, we have demonstrated that IMAC is involved in NADH/ROS regulation of I_{Na} [16].

Other mitochondrial ion channels may also influence arrhythmias. The mitochondrial Ca^{2+} uniporter modulates mitochondrial Ca^{2+} homeostasis [118]. Mitochondrial Ca^{2+} signaling is important in the control of fundamental energy functions including energy metabolism and apoptotic cell death [119]. The Ca^{2+} influx, mediated by the mitochondrial Ca^{2+} uniporter, is associated with opening the mitochondrial membrane permeability transition pore [120], and impairment in intracellular ion homeostasis and mitochondrial function have been implicated in the development of cardiomyopathy and arrhythmia [121].

Improper regulation of mitochondrial ion channels can lead to metabolic stress that produces local regions of elevated ROS, triggering slow oscillations of ROS, the $\Delta\Psi_m$, NADH and ATP. ROS increase the probability of opening the inner mitochondrial membrane ion channels, depolarize the $\Delta\Psi_m$, and result in a reduction of the ATP/ADP ratio. This activates repetitive and self-sustaining oscillations of the sarcK_{ATP} channels. Additionally, increases in NADH would be expected to cause decreases in Na^+ current, resulting in the formation of spatially and temporally varying islands of inexcitability that can lead to arrhythmias.

C. Intracellular Ca^{2+} handling and oxidative stress in arrhythmia

In the process of EC coupling, cardiac contraction is triggered by membrane depolarization followed by the AP and subsequent Ca^{2+} entry through L-type Ca^{2+} channels. This initial Ca^{2+} influx drives the SR Ca^{2+} release via ryanodine receptor (RyR) opening, so called Ca^{2+}-induced Ca^{2+} release (CICR). In contrast, cardiac relaxation is mediated by Ca^{2+} removal through the reuptake of the cytosolic Ca^{2+} into sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) and extrusion by Na^+-Ca^{2+} exchanger (NCX) [122]. Abnormal Ca^{2+} handling contributes to a variety of pathophysiological disease conditions, such as HF and hypertrophy. Many reports demonstrated that alterations in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) can also affect arrhythmias through altered ion channel and gap junction conductances [123, 124].

Metabolic stress, intracellular acidification, reduction in ATP, and a rise in ADP concentrations have been shown to increase cytoplasmic free $[Ca^{2+}]_i$ and inhibit both SR Ca^{2+} uptake and release [125]. Among 5 subunits that make up the voltage-gated, dihydropyridine-sensitive L-type Ca^{2+} channel ($I_{Ca,L}$), the α_{1C} pore-forming subunit, is redox sensitive. $I_{Ca,L}$ decreases when exposed to thiol oxidizing agents (e.g. thimerosal), and this decrease is reversed by dithiothreitol (DTT) [126]. Furthermore, in ferret ventricular myocytes, redox-sensitive thiols in L-type Ca^{2+} channel diminish $I_{Ca,L}$ under oxidizing conditions, and this mechanism is regulated by NO and S-nitrosothiols [127]. Metabolic inhibition with increased $[Ca^{2+}]_i$ causes a reduction of the amplitude of $I_{Ca,L}$ in the ventricular myocytes of guinea pigs, rats, and rabbits, which contributes to shortening of the APD and an increased potential of reentrant arrhythmias [128-132].

In cardiac muscle, SERCA2a removes cytosolic Ca^{2+} into SR. SERCA also contains redox-sensitive cysteine residues in the active site, and oxidative stress impairs SERCA activity by oxidation of these sulfhydryl groups [133]. Reducing agents (e.g. DTT and GSH) preserve SERCA activity in the face of oxidative stress [134]. SERCA dysregulation is accompanied by concomitant AP prolongation [135], a potential source of triggered activity.

RyR is involved in cardiac E-C coupling by allowing SR Ca^{2+} release during CICR. RyR2 is the cardiac muscle specific isoform. RyRs consist of four large subunits, comprised of numerous regulatory subunits including calmodulin, the FK506-binding protein FKBP12.6 (also known as calstabin2), PKA, Ca^{2+}/calmodulin kinase II (CaMKII), protein phosphatases 1 and 2A, phosphodiesterase, junctin, triadin, and calsequestrin [136]. A number of studies have shown that various ROS mediate RyR sulfhydryl oxidation (reviewed in ref [137]). An altered redox balance leads to reduced Ca^{2+} transients and enhanced SR Ca^{2+} leak in the failing heart, and these changes can be reversed by reducing agents [138]. This leak may be mediated by oxidative alteration of RyR [139]. Among the 89 cysteine residues of RyR, there are a few sulfhydryl groups that seem to mediate redox-sensitivity of the channel [140]. Free SH oxidizing reagents, 2.2′-dithiodipyridine (DTDP) or 5.5′-dithio-bis(2-nitrobenzoic acid) (DTNB), activate the RyR channel by formation of disulfide bonds within the RyR complex [141]. Oxidized GSSG stimulates RyR2 activity by decreasing calmodulin binding affinity [141, 142]. Exposure to superoxide stimulates RyR2 causing pro-arrhythmogenic SR Ca^{2+} releases [42]. Zima et al. also show that cytosolic NADH inhibited cardiac SR Ca^{2+} release channels, while NAD^+ had activating effects on this channel [143]. Clinical significance of these RyR changes is reinforced by the observation that genetic alterations in RyR2 are linked to an autosomal-dominant form of inherited cardiac arrhythmias, known as catecholaminergic polymorphic VT [144]. Catecholaminergic polymorphic VT-associated variants sensitize RyR2 to activation by cytosolic Ca^{2+} [145].

Another important Ca^{2+} handling protein, NCX, is also sensitive to redox-sensitive regulation. NCX is the main Ca^{2+} efflux pathway, removing intracellular Ca^{2+} using the Na^+ transmembrane gradient. Typically delayed afterdepolarization-type triggered activity is induced when high intracellular Ca^{2+} promotes NCX activity and associated depolarizing currents (reviewed in [136]). Consistent with this, upregulated of NCX enhances delayed afterdepolarizations [146]. Moreover, the reverse mode of NCX can lead to Ca^{2+} overload

and potentially to early afterdepolarizations [147]. Hinata and colleagues demonstrated that H_2O_2 exposure stimulated NCX activity by activating mitogen-activated protein kinase and Src tyrosine kinase signaling pathways [148].

Altered Ca^{2+} homeostasis can contribute to arrhythmias by ROS-dependent CaMKII activation that enhances late I_{Na}, leading to cellular Na^+ and Ca^{2+} overload and afterdepolarizations [149]. Morita and colleagues demonstrated that oxidative stress promotes early afterdepolarizations and triggered activity leading to VT and VF in aged fibrotic rat and rabbit ventricular myocytes. These arrhythmias were suppressed by the antioxidant, NAC, and CaMKII inhibitor, consistent with the oxidative activation of CaMKII [150, 151].

Stretch activated channels may play a role in redox-dependent Ca^{2+} alterations and arrhythmogenesis. The combination of mechanical stretch and low level oxidative stress triggers Ca^{2+} overload and prolong APD through premature beat resulting early afterdepolarizations [152]. This may explain the reduction of ventricular arrhythmias with intra-aortic balloon counterpulsation [153, 154].

D. Potassium channels in arrhythmogenesis

The metabolic stress and oxidative stress have been shown to affect repolarizing K^+ currents, potentially causing arrhythmias. APD is regulated by a balance between inward depolarizing and K^+ outward currents. The importance of ADP for arrhythmogenesis is highlighted by the large number of hERG (human *ether-a-go-go*-related gene) K^+ channel mutations associated with long-QT syndrome and the case of congenital short QT syndrome with a gain-of-function in the delayed rectifier K^+ channel, I_{Kr} [155]. Oxidative stress levels result in decreased hERG protein levels and arrhythmias in diabetic rabbits, and the abnormal QT prolongation is restored by the insulin treatment [156]. Direct treatment with H_2O_2 triggers an initial hERG channel activation and subsequent acceleration of channel deactivation, increasing the risks of ectopy [157]. This effect is mediated by thiol oxidation [158, 159].

Another important outward K^+ current is the transient outward current (I_{to}), which is responsible for the rapid repolarization phase (phase 1) in ventricular AP. This K^+ channel current is composed of several α subunit isoforms, Kv1.4, Kv4.2, and Kv4.3 and the β subunits, KChIP2 and Kvβ1.2. Downregulation of I_{to} has been shown in the diabetic heart, and this change could be reversed by the insulin treatment [160]. Recently, Marionneau and colleagues suggested peroxisome proliferator-activated receptor alpha (PPARα), a key regulator of fatty acid and glucose metabolism, mediated most of this change in current [161]. Nevertheless, a increase ROS induced downregulation of I_{to} in diabetic heart, and this was reversed by reduced antioxidant, GSH [160]. The channel subunit, K_vβ1.2, has been implicated in the redox control of K^+ channels [162, 163].

Redox-dependent regulation has been reported in the inward rectifying K^+ channel, I_{K1}. S-nitrosylation of Cys76 residue in α subunit of Kir2.1 encoding this current increases the channel opening probability. Alternatively, NO increases the amplitude of this current [164].

Since this channel sets resting membrane potential and influences APD, redox-dependent changes would be expect to influence arrhythmic risk.

E. Gap Junction Remodeling

ROS has been implicated in gap junction remodeling. Impulse propagation is dependent on several factors, including intrinsic membrane excitability and cell-to-cell coupling. Cardiac cells are connected by gap junction channels that are low resistance pathways for electrical coupling of myocytes and are located within the intercalated disks [165]. Gap junction channels are made of Cxs, a family of proteins designated by molecular weight [166, 167]. In the adult heart, ventricular gap junctions are predominantly formed by Cx43 protein, and atrial gap junctions are formed mainly by Cx40 and Cx43 [167].

Abnormalities in gap junctional communication have been shown in cardiac ischemia [168, 169]. Intracellular acidosis associated with cardiac ischemia causes the direct interaction of the carboxyl terminal domain of Cx43 with the second half of the cytoplasmic loop of Cx43. This in turn results in closure of the channel [170], a reduction in conduction velocity in the ischemic area. In addition, cardiac ischemia affects the balance of protein kinase and phosphatases in a way that favors the dephosphorylation of Cx43, which results in the impairment of gap junction [171-173]. Lateralization involves the diffusion of Cx43 away from the intercalated disks and toward the lateral myocyte walls. A significant portion of Cx43 is lateralized and is thus nonfunctional after cardiac ischemia [169, 174] which can result in dysfunction of gap junctions. These changes are associated with increased arrhythmic risk.

In another mechanism that may alter gap junctions and arrhythmic risk, Kieken and colleagues showed that the phosphorylated form of c-Src tyrosine kinase competes with Cx43 to bind to the scaffolding protein zonula occludens-1 at the intercalated disks and that elevated levels of phosphorylated c-Src at the border zone of ischemia reduce the Cx43 [169]. The c-Src tyrosine kinase has been linked primarily to tumor growth, and the inhibition of c-Src has been shown to be effective in controlling cancers [175-179]. Elevated levels of ROS and AngII can result in the upregulation of c-Src [180-182]. In cardiac myocytes, Aikawa and colleagues have shown that treatment with H_2O_2 activates Src family tyrosine kinases in a concentration dependent manner [181]. Also, it has been shown that, in a rat model of Adult Respiratory Distress Syndrome, oxidative stress results in increased level of c-Src [182]. Some studies support the existence of a positive feedback loop between ROS and c-Src in which increase in ROS upregulates c-Src and the c-Src upregulation increases oxidative stress further [183].

The homozygous cardiac angiotensin converting enzyme (ACE) gene-targeted mice (ACE8/8) show normal LV function and structure [92]. Nevertheless, these animals show a high rate of SCD due to VT, VF and bradyarrhythmia. AngII results in elevation of ROS [69, 184] and oxidative stress in the cardiac tissue of the ACE8/8 [185]. Western blotting of the ventricular tissue of the ACE8/8 mice shows higher level of c-Src and a reduction in the total amount of Cx43 to 30% of controls. Administration of PP1 ((1-(1, 1-Dimethylethyl)-1-(4-methylphenyl)-1H-pyrazolo [3, 4-d] pyrimidin-4-amine), a specific c-Src inhibitor, reduced the total and phosphorylated form of c-Src, improved the total amount of Cx43 and

corrected the gap junctional conduction impairment. These effects of PP1 were associated with correction of phenotype and prevention of sudden arrhythmic death. Since oxidative stress activates c-Src, c-Src downregulates Cx43, and Cx43 levels are inversely related to arrhythmic risk, this represents another ROS-dependent mechanism that may increase arrhythmic risk.

4. Therapeutic implications

Conventional antiarrhythmic drugs target ion channels and reduce ion currents. Nevertheless, in many cases, the targeted ion channels are already downregulated. The role of ROS in arrhythmogenesis, as outlined above, opens new and potentially safer therapeutic options to treat arrhythmias using antioxidants. Administration of either GSH or *N*-acethylcysteine significantly reduces reperfusion arrhythmias [186]. Additionally, altering ratios of GSH/GSSH can affect the $\Delta\Psi_m$ and the occurrence of arrhythmias [113]. ROS-mediated collapses of the $\Delta\Psi_m$ is prevented by the addition of exogenous GSH [187]. Ascorbate (vitamin C) reduces the incidence of atrial pacing-induced peroxynitrite formation as well as postoperative AF [72]. Vitamin E analogues scavenge free radicals and reduce the incidence of ischemia/reperfusion-induced VF [188]. Another synthetic ROS scavenger, 6,6-methylene bis 2,2-dimethyl-4-methane sulphonic acid: Na-1,2-dihydroquinoline (MTDQ-A), significantly reduces the incidence of VF after myocardial infarction following coronary ligation in a dog [189].

Nevertheless, general antioxidant strategies have not always been successful in antiarrhythmic therapies. Explanations for the strong association of oxidative stress and arrhythmia but a more qualified success of antioxidant strategies to prevent arrhythmias may be explained by the idea that redox stress is compartmentalized. As we discussed above, mitochondrial oxidative stress contributes to a wide range of damage in cardiac metabolism and ion homeostasis. Therefore, reducing mitochondria oxidative stress could be a more effective anti-oxidant approach. Indeed, several mitochondria-targeted ROS scavengers have been developed and are ripe for study of their potential anti-arrhythmic properties [112, 114, 190-192].

Strategies targeting mitochondrial ROS release pathways may be antiarrhythmic [114, 193]. IMAC inhibitors, DIDS and PK11195, can prevent the cell-wide accumulation of ROS and reversible collapses in the $\Delta\Psi_m$ [114, 193]. Furthermore, 4′chlorodiazepam, a mitochondrial benzodiazepine receptor modulator, can prevent reperfusion arrhythmias in isolated rabbit heart [112]. This inhibitor, as well as DIDS and PK11195, can prevent the I_{Na} decrease induced by elevated NADH and ROS [16]. Additionally, other mitochondrial targeted antioxidants such as peptides, SS-02 (Dmt-D-Arg-Phe-Lys-NH2), SS-31 (D-Arg-Dmt-Lys-Phe-NH2) and SS-20 (Phe-D-Arg-Phe-Lys-NH2) are also effective in ischemia-reperfusion [194, 195]. Arrhythmias were significantly reduced in ischemia-reperfusion injured rat hearts when treated SS-02 and SS-31 directly before reperfusion [196, 197].

The mitochondrial anti-oxidant, MitoQ, is targeted to the mitochondria by covalent conjugation with a lipophilic triphenylphosphonium cation. As previously discussed, CoQ (ubiquinone) accepts electrons from complex I or II, and donates to complex II by the

formation of reduced product, ubiquinol [191]. The protective effects of MitoQ have been demonstrated by the administration to rats in their drinking water [191]. Under ischemia-reperfusion injury, MitoQ shows significant protection against heart dysfunction, tissue damage, and mitochondrial dysfunction [191]. A similar study also has shown that MitoQ has the protective effects on cardiac ischemia-reperfusion injury [190]. MitoQ shows a protective effect against an increased blood pressure in a hypertensive rat model, which has mitochondrial oxidative damage in endothelial cells [192]. Therefore, it is likely to have antiarrhythmic properties.

Taken together, these findings suggest that reduced cardiac oxidation may be a promising therapeutic strategy for treatment of arrhythmias.

5. Summary

Oxidative stress is highly associated with cardiac arrhythmogenesis. Among other sources, altered mitochondrial metabolism can lead to ROS. Targeting oxidative changes, especially mediated by mitochondria, may represent a new strategy to prevent arrhythmias that could be safer than the conventional ion channel blockers used now.

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Abbreviations

$\Delta\Psi_m$	mitochondrial membrane potential
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
AF	atrial fibrillation
AngII	angiotensin II
AP	action potential
ATP	adenosine-5′-triphosphate
$[Ca^{2+}]_i$	intracellular Ca^{2+} concentration
CICR	Ca^{2+}-induced Ca^{2+} release CICR
Cx	connexins
DTT	dithiothreitol
E-C coupling	excitation-contraction coupling
ETC	electron transport chain
GSH	glutathione
GPD1-L	glycerol-3-phosphate dehydrogenase 1-like protein

hERG	human *ether-a-go-go*-related gene
HF	heart failure
HIFα	Hypoxia inducing factor-1α
IMAC	inner membrane anion channel
I_{Na}	sodium current
I_{Kr}	delayed rapid rectifier K^+ current
I_{Ks}	delayed slow rectifier K^+ current
MAP	monophasic action potential
mitoK_{ATP}	mitochondrial ATP-sensitive potassium channel
NAD	nicotinamide adenine dinucleotide
$Na_v1.5$	cardiac sodium channel
NCX	Na^+-Ca^{2+} exchanger
NO	nitric oxide
NOS	nitric oxide synthase
PES	programmed electrical stimulation
PK	protein kinase
RAS	renin-angiotensin system
ROS	reactive oxygen species
RyR	ryanodine receptor
sarcK_{ATP}	sarcolemmal ATP-sensitive potassium channel
SCD	sudden cardiac death
SCN5A	cardiac sodium channel gene
SERCA	sarcoplasmic reticulum Ca^{2+}-ATPase
SR	sarcoplasmic reticulum
TCA	tricarboxylic acid
TdP	torsades de pointes
VT	ventricular tachycardia

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Highlights

- Cardiomyopathies are associated with metabolic stress, oxidative stress, and arrhythmic risk.
- Oxidative stress alters ion channels, Ca^{2+} handling, and gap junctions, possibly explaining the arrhythmic risk
- Anti-oxidants may be useful antiarrhythmic drugs.

Highlights

ROS mediate arrhythmogenesis through the alteration of ion homeostasis and structural remodeling.

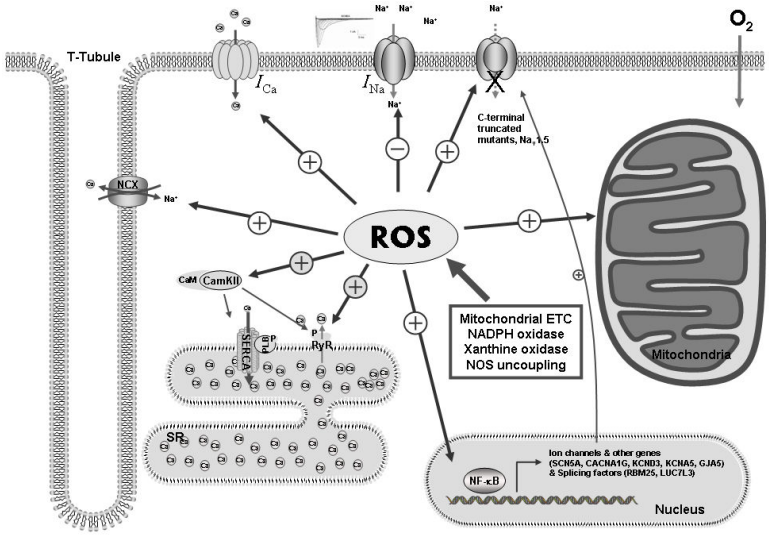

Figure 1.

Schematic illustration of ROS-mediated ion channel alterations. ROS are generated from mitochondrial ETC electron leakage, NADPH oxidases, xanthine oxidase and NOS uncoupling in the heart. ROS induces functional and structural alteration of $Na_v1.5$ by both transcriptional and posttranslational mechanisms. Elevated ROS mediates SERCA inhibition, enhanced SR Ca^{2+} release from RyR, enhanced inward L-type Ca^{2+} current, and increased NCX activity to increase intracellular Ca^{2+} level. ROS-mediated CaMKII activation stimulates hyperphosphorylation of RyR, resulting SR Ca^{2+} leak. In mitochondria, stimulated ROS are release by the IMAC and the membrane permeability transition pore, causing K_{ATP} activation, the mitochondrial calcium uniporter activity, and NADH accumulation. This ROS-mediated network is likely to contribute to the pathogenesis of arrhythmias.

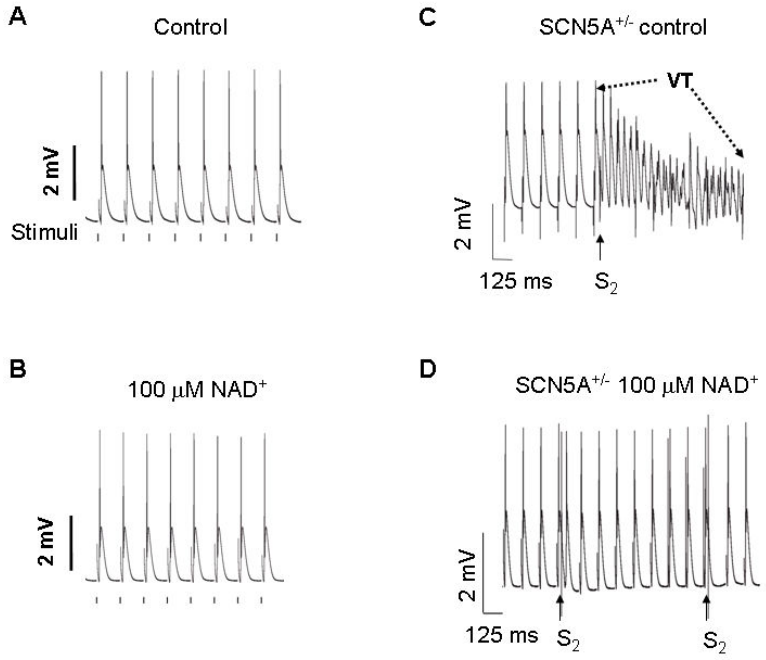

Figure 2.

The effects of altering NAD(H) on arrhythmic risk in a mouse model of reduced Na^+ current. Representative traces of MAPs from LV epicardium of (A) Langendorff-perfused $SCN5A^{+/-}$ heart during standard pacing at BCL of 125 ms in the control condition. Vertical lines below the monophasic action potentials (MAPs) represent the times when electrical stimulations were delivered. (B) MAPs after 20 min of perfusion with 100 μmol/L $[NAD^+]_o$. (C) Representative MAPs recorded during programmed electrical stimulation (PES) showing PES-induced ventricular tachycardia in $SCN5A^{+/-}$ hearts under control condition. The final six paced beats at 125 BCL (S_1) were followed by an extra stimulus (S_2) delivered at a S_1-S_2 interval of 42 ms. PES induced a polymorphic ventricular tachycardia of frequency, 20-40 Hz, sustained for ≈19 seconds. (D) Representative trace of PES-induced MAP recording in same $SCN5A^{+/-}$ heart after 20 min perfusion with 100 μM $[NAD^+]_o$. S_2 stimuli delivered at a 35 ms S_1-S_2 interval produced a single MAP but failed to induce any arrhythmia. (Modified from reference [60])

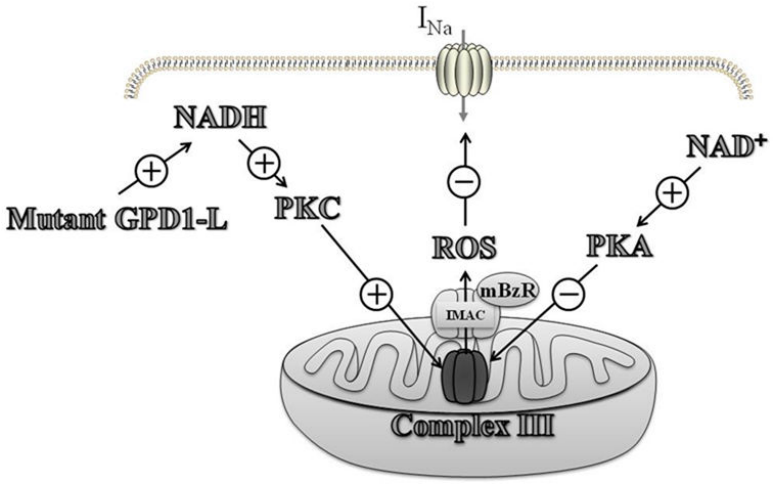

Figure 3.

The illustration of the effects of mitochondrial ROS induced by NADH on the I_{Na}. A proposed signaling pathway by which mutant GPD1-L and NADH downregulate $Na_v1.5$ by causing PKC activation and ROS overproduction from the mitochondrial ETC. ROS are released from the mitochondria by the IMAC, which is modulated by the mitochondrial benzodiazepine receptor. NAD^+ inhibits mitochondrial ROS overproduction through PKA activation followed by the upregulation of $Na_v1.5$. (Modified from reference [16])