REVIEWS

Mitochondrial targets for arrhythmia suppression: is there a role for pharmacological intervention?

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Abstract Mitochondrial dysfunction is a hallmark of common cardiovascular disorders, including ischemia–reperfusion injury, hypertrophy, heart failure, and diabetes mellitus. While the role of the mitochondrial network in regulating energy production and cell death pathways is well established, its active control of other critical cellular functions, including excitation–contraction coupling and excitability, is less understood. The purpose of this focused review article is to highlight the growing mechanistic link between mitochondrial dysfunction and arrhythmogenesis. The goal is not to provide a comprehensive listing of all factors by which mitochondrial bioenergetics and altered cellular redox status affect ion channel function but rather to focus on one central mechanism of arrhythmogenesis which arises from a mitochondrial origin. In doing so, we discuss the role of mitochondrial targets for suppressing arrhythmias through this mechanism.

Abbreviations

I/R	Ischemia–reperfusion injury
LVH	Left ventricular hypertrophy
HF	Heart failure
MI	Myocardial infarction
AP	Action potential
APD	Action potential duration
ETC	Electron transport chain
ATP	Adenine triphosphate
DYm	Mitochondrial membrane potential
ROS	Reactive oxygen species

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H_2O_2	Hydrogen peroxide
O_2^-	Superoxide anion
RIRR	ROS-induced ROS release
OS	Oxidative stress
sarcK$_{ATP}$	Sarcolemmal ATP-sensitive potassium channels
mPTP	Mitochondrial permeability transition pore
CsA	Cyclosporine-A
IMAC	Inner membrane anion channel
4′-Cl-DZP	4′-Chlorodiazepam (IMAC blocker)
mBzR	Mitochondrial benzodiazepine receptor

1 Introduction

Mitochondria are critical determinants of cardiac function in health and disease. They are the main sources of adenine triphosphate (ATP), the energy currency used by myocytes to fulfill their contractile duties. In addition, mitochondria generate reactive oxygen species (ROS) which are very important cell signaling molecules [1]. A key metric of mitochondrial function is the mitochondrial membrane potential ($\Delta\Psi_m$), a large (~200 mV) voltage gradient that exists across the inner mitochondrial membrane and that forms the proton-motive force used by mitochondria to shuttle electrons down the ETC in a series of highly orchestrated chemical reactions that ultimately result in ATP synthesis and ROS generation [1]. Under normal physiological conditions, $\Delta\Psi_m$ is highly regulated such that both ATP synthesis and ROS production are maintained within a physiological range that matches energy supply to demand while preventing excessive ROS generation [1]. In the context of various cardiovascular disorders, ROS production can exceed the ability of myocytes and other cell types to detoxify these inherently unstable and reactive molecules, resulting in oxidative stress (OS). Indeed, OS serves as a

root cause and a consequence of mitochondrial dysfunction in response to a wide range of cardiovascular insults which ultimately culminate in myocardial infarction (MI), heart failure (HF), and arrhythmias (Fig. 1).

In this review article, we highlight the growing link between mitochondrial dysfunction and arrhythmogenesis by focusing on a central mechanism of OS known as ROS-induced ROS release (RIRR). Based on our growing appreciation of the biophysical properties and arrhythmic consequences of this autocatalytic process, we discuss therapeutic options to suppress arrhythmias by targeting mitochondrial pathways that modulate RIRR. We describe pharmacological interventions that may ultimately have widespread applications to the management of clinically relevant cardiac disorders, ranging from ischemia–reperfusion (I/R) injury to left ventricular hypertrophy (LVH) and HF.

Before proceeding to the main subject of this review, however, it is important to emphasize that the pro-arrhythmic effects of ROS are complex and multifactorial. Indeed, different cellular sources and types of ROS exert potent electrophysiological (EP) effects on a host of ion channels, exchangers, and pumps. For example, increased oxidation decreases sodium [2] and potassium [3] current densities, activates sarcolemmal

KATP (sarcK$_{ATP}$) channels [4], alters the inactivation kinetics of L-type calcium and sodium channels, increases ryanodine receptor calcium leak [5], modulates intracellular calcium cycling, disrupts forward trafficking of Cx43 to the intercalated disc, and alters gap junction function [6]. The profound local control of numerous membrane-bound and intracellular proteins by ROS is expected to impact cell-to-cell coupling [6], conduction [2], repolarization [3], alternans [7], calcium-mediated triggers [5], and arrhythmias through multiple mechanisms that are independent of RIRR and associated mitochondrial dysfunction (Fig. 1, dotted line). In this review article, we restrict our discussion to one important facet of ROS-mediated arrhythmias that stems from a mitochondrial origin. This allows us to provide a detailed view of these arrhythmias and to propose therapeutic interventions based on their underlying mechanism. For a general discussion of ion channel regulation by metabolic factors and the redox state of the myocyte, the reader is referred to excellent reviews on this broad topic [8].

2 ROS-induced ROS release as a mechanism of oxidative stress

Zorov et al. [9] advanced the notion of RIRR to explain how local ROS injury within a discrete region of a cardiomyocyte can rapidly accumulate across a critical mass of the mitochondrial network to cause cellular OS. In these studies, RIRR was described as a fundamental mechanism by which cardiac mitochondria respond to elevated ROS levels by stimulating endogenous ROS production in a regenerative, autocatalytic process that ultimately results in cellular dysfunction and death.

Distinct modes of RIRR have been postulated based on their dependence on various mitochondrial ion channels [10]. Specifically, Zorov et al. [9] demonstrated a convincing relationship between the destabilization of $\Delta\Psi_m$ upon mitochondrial oxidation and the induction of the mitochondrial permeability transition which causes apoptosis [9]. On the other hand, studies by Aon et al. [11] provided strong evidence in support of the inner membrane anion channel (IMAC) as a mediator of RIRR and associated metabolic instabilities. In these studies, photo-induced oxidation of a discrete region within the cardiac myocyte unleashed a regenerative process of RIRR that was dependent on IMAC activation. Once a threshold level of ROS was exceeded across a critical mass of the mitochondrial network, sustained $\Delta\Psi_m$ oscillations were initiated [12, 13]. As will be discussed next, these mitochondrial oscillations can result in cellular action potential (AP) oscillations via cyclical activation of sarcK$_{ATP}$ channels providing compelling evidence of a mechanistic link between mitochondrial instability and cellular electrical dysfunction.
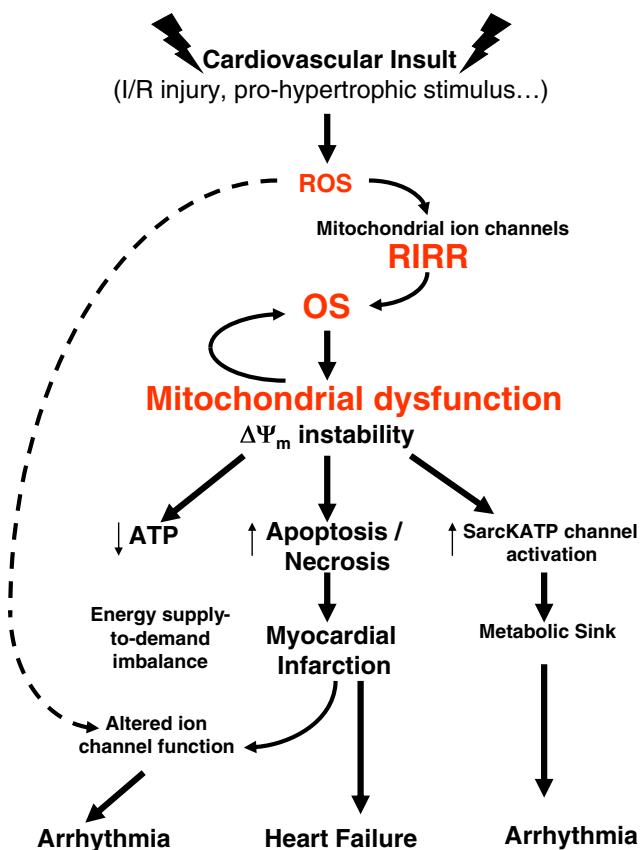

Fig. 1 Schematic illustrating the central role of reactive oxygen species and mitochondrial dysfunction in the pathogenesis of common cardiovascular disorders

3 Mechanistic link between mitochondrial and cellular EP instability

The functional significance of metabolic oscillations was demonstrated by studies in which simultaneous measurements of $\Delta\Psi_m$ (using two-photon imaging) and the cellular action potential (using patch clamp recording in current clamp mode) were performed [11]. Photo-induced oxidation caused RIRR, $\Delta\Psi_m$ depolarization, and oscillatory metabolic and EP behavior. Importantly, these studies revealed that cyclical oscillations of the AP were in phase with $\Delta\Psi_m$ oscillations [11]. Specifically, during episodes of $\Delta\Psi_m$ collapse, the AP also collapsed to an inexcitable state. Since AP recovery always coincided with, and therefore likely depended upon, $\Delta\Psi_m$ recovery, these findings provided evidence for a profound mitochondrial control of cellular excitability, at least at the isolated myocyte level [11]. The ionic basis for these cellular AP oscillations involved sarcK$_{ATP}$ channels, which are known to link membrane excitability to metabolism. Indeed, cellular AP oscillations were driven by "out-of-phase" sarcK$_{ATP}$ current activation during the process of RIRR [11].

4 Sarcolemmal K$_{ATP}$ channels as downstream effectors of RIRR-mediated arrhythmias

The dynamic relationship between sarcK$_{ATP}$ channel activation and the metabolic status of the cardiomyocyte was first observed by O'Rourke and colleagues [14]. While sarcK$_{ATP}$ channel activation protects the viability of ischemic tissue by limiting intracellular calcium cycling and force generation during periods of reduced energy supply, increased potassium (K$^+$) conductance through these channels may predispose to electrical dysfunction and arrhythmias. Due to their abundance in the plasma membrane, the opening of sarcK$_{ATP}$ channels causes rapid AP shortening, loss of intracellular K$^+$, and reduction in myocyte excitability. The pro-arrhythmic potential of sarcK$_{ATP}$ channel activation could be attributed to shortening of the AP duration (APD), and therefore the cardiac wavelength, at a time when calcium-mediated triggers are known to arise. Moreover, the opening of sarcK$_{ATP}$ channels may create heterogeneous current sinks which can slow or block conduction wavefronts in local regions where the open probability of sarcK$_{ATP}$ channels is high (i.e., where the energetic status of the cell is compromised), a phenomenon coined "metabolic sink" by O'Rourke and colleagues [15, 16].

While the pro-arrhythmic potential of sarcK$_{ATP}$ channel activation has been confirmed in multiple studies, the use of glibenclamide as a channel blocker has yielded mixed results in terms of anti-arrhythmic efficacy, with some studies emphasizing adverse effects [17]. Notably, sarcK$_{ATP}$ channel blockade with glibenclamide failed to delay the onset of inexcitability during late ischemia or the initiation of arrhythmias upon reperfusion in the *ex vivo* perfused guinea pig heart [15]. This highlights the importance of understanding the upstream factors (i.e., mitochondrial ion channels) that drive the pathological opening of sarcK$_{ATP}$ channels during RIRR.

5 Mitochondrial ion channels are root causes of and appropriate targets for arrhythmias

A rich diversity of ion channels and transporters has been discovered in the inner and outer membranes of mitochondria [1]. Of note are various mitochondrial pathways that modulate bioenergetics, apoptosis, cardioprotection, and calcium homeostasis, including components of the mitochondrial permeability transition pore (mPTP), the IMAC, mitochondrial calcium-dependent channels (mitoKCa), the mitochondrial calcium uniporter (MCU) and its regulatory subunit (MICU1) [18], the mitochondrial ryanodine receptor (mRYR1) [19], the sodium–calcium exchanger (mNCX), and uncoupling proteins (UCP) [20]. In this review article, we focus on the mPTP and IMAC because of their established roles in RIRR [21] and associated dysfunction (Fig. 2). The interested reader is referred to excellent reviews that cover mitochondrial ion channel targets in a more comprehensive manner [1, 22].
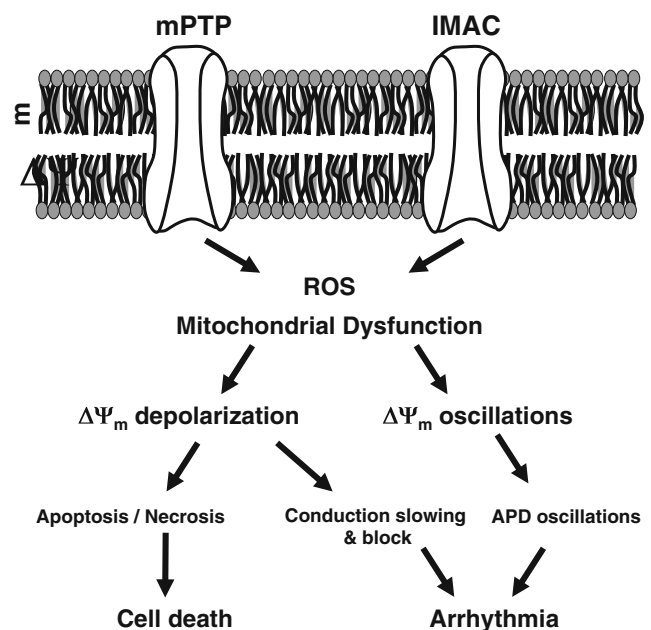

Fig. 2 Key mitochondrial ion channels which have been implicated in the amplification of ROS levels within the cardiomyocyte through the regenerative process of mitochondrial ROS-induced ROS release. A general scheme is presented by which activation of these channels can destabilize the mitochondrial membrane potential leading to cell death or arrhythmias through multiple mechanisms

5.1 The mitochondrial permeability transition pore

The mPTP has received considerable attention as a potential therapeutic target because of its impact on the activation of pathways leading to cellular necrosis and apoptosis. Experimentally, mPTP blockade with cyclosporine-A (CsA) attenuates MI, left ventricular dysfunction, cardiomyocyte death, and I/R injury. Clinically, administration of CsA prior to percutaneous coronary intervention decreased the extent of short-term injury. This highlighted the translatability of this promising strategy [23], which may exert potent cardioprotective effects directly through mPTP inhibition or indirectly via calcineurin signaling.

Although the role of the mPTP in cell death is well established, its involvement in the generation of acute arrhythmias remains highly controversial. While some studies showed that mPTP blockade exerted moderate protection against arrhythmias, other studies confirmed a lack of protection in rat [24], guinea pig [15], and rabbit [25] hearts. Moreover, application of a CsA bolus immediately before the stent procedure did not abrogate the incidence of ventricular fibrillation in humans [23]. Failure of mPTP blockade to protect against arrhythmias is also supported by mechanistic studies in which $\Delta\Psi_m$ depolarization caused by substrate deprivation or photo-induced oxidation was not prevented by CsA [11]. However, it is important to note that by reducing infarct size following an ischemic insult, mPTP blockade is indeed cardioprotective [26] and hence may suppress scar-related arrhythmias that are associated with healed MI. Also, by inhibiting myocyte loss and improving left ventricular function, this strategy may confer an anti-arrhythmic benefit through long-term mechano-electrical feedback which may hinder or reverse disease progression. Direct evidence for this hypothesis, however, remains lacking.

5.2 The IMAC

The existence of IMAC has been confirmed by numerous investigators who documented its role in mediating anion efflux from energized mitochondria [27]. Although the exact structure and molecular identity of IMAC remain elusive, the tight regulation of this channel by benzodiazepine compounds such as 4′-chlorodiazepam (4′-Cl-DZP) suggests a strong association between a partially anion-selective pore-forming subunit in the inner membrane and a peripheral benzodiazepine receptor in the outer mitochondrial membrane (i.e., mitochondrial benzodiazepine receptor, mBzR) [27].

The importance of IMAC in modulating RIRR was documented by studies in which IMAC ligands effectively prevented pathological $\Delta\Psi_m$ oscillations in isolated cardiomyocytes [11]. Importantly, blocking $\Delta\Psi_m$ oscillations by targeting the IMAC also inhibited transmembrane voltage oscillations and prevented myocyte inexcitability [11]. This provided indirect evidence that targeting the IMAC may be an effective strategy for preventing electrical instability in an artificial *in vitro* model system of stress-induced RIRR. In what follows, we highlight studies that have extended this intriguing notion to the intact heart.

6 Mitochondrial function in the intact heart: metabolic sink as an arrhythmia mechanism

Despite major advances in our understanding of mitochondrial biochemistry at the subcellular/molecular levels, the pathophysiological consequences of mitochondrial dysfunction at the level of the intact heart remain unclear. Since mitochondrial function of individual cells is highly influenced by network properties, it is critical to investigate mitochondrial function within the milieu of the intact heart where tissue-network properties may modulate metabolic function. In support of this notion, we and others have found that mitochondria form functionally interactive networks across broad regions of the heart that may determine the global cardiac response to OS [28–30].

For example, we recently extended the concept of RIRR from a subcellular phenomenon to one occurring at the organ level, in which functional electrical instability and arrhythmias can be directly examined. This allowed us to further investigate the biophysical features of RIRR at the tissue-network level and assess mitochondrial channel mechanisms [31]. We found that a short episode of OS produced by high but not low concentrations of H_2O_2 always elicited two distinct superoxide (O_2^-) peaks that can be readily distinguished by their relative timing, maximum amplitude, and persistence after the triggering insult [31]. As such, these findings are consistent with cellular studies of RIRR. Notably, since the large secondary O_2^- peak (P2) was always initiated following, not during, the initial insult, it most likely reflects the capacity of the myocardium to form a regenerative RIRR process that can outlast the initial perturbation [31]. We examined the functional significance of RIRR in terms of electrical instability and found that electrical rhythm in all hearts that exhibited P2 (and therefore RIRR) also exhibited spontaneous onset of sustained VT/VF. In contrast, hearts that did not exhibit P2 were protected against sustained arrhythmias [31]. As such, these findings extended previous *in vitro* studies by implicating RIRR in arrhythmia propensity and severity.

As mentioned above the concept of metabolic sink as a potential arrhythmia mechanism arising from RIRR requires a spatially heterogeneous mitochondrial (specifically $\Delta\Psi_m$) substrate which may underlie local changes in sarcK$_{ATP}$ current density creating local areas of depressed excitability and conduction bock [15, 16]. In what follows, we describe recent studies that have provided credence to the concept of

the metabolic sink by linking unstable mitochondrial properties in the intact heart to arrhythmias associated with I/R injury and LVH.

7 Mitochondrial dysfunction in ischemia–reperfusion injury

Most sudden cardiac deaths occur in patients with coronary artery disease (CAD) and associated left ventricular dysfunction. Epicardial coronary artery abnormalities resulting in acute or chronic ischemic insults account for up to 80 % of clinical arrhythmias. Indeed, morbidity and mortality in patients with CAD and associated I/R injury remain significantly high despite our best efforts to ameliorate them. Mechanistically, I/R injury arises from OS-induced mitochondrial dysfunction, which leads to myocyte death and MI (Figs. 1 and 2) [32, 33]. Another hallmark of I/R injury is a marked propensity for lethal arrhythmias. As will be discussed next, understanding the spatio-temporal dynamics of mitochondrial function in the intact heart during I/R injury has presented us with an opportunity to combat arrhythmias by targeting relevant mitochondrial pathways that give rise to these arrhythmias.

7.1 $\Delta\Psi_m$ depolarization during ischemia

In isolated myocytes, $\Delta\Psi_m$ depolarization is a critical event that promotes cellular dysfunction and, if excessive, death through necrosis or apoptosis. To investigate the functional consequences of $\Delta\Psi_m$ depolarization during ischemia in intact myocardium and its potential relationship to arrhythmias, we have used a semiquantitative approach of optical $\Delta\Psi_m$ imaging in the *ex vivo* perfused rat heart. While on average $\Delta\Psi_m$ was depolarized by ~60 % in response to global no-flow ischemia to the rat myocardium, changes in $\Delta\Psi_m$ were both spatially and temporally heterogeneous. Well defined zones of polarized and depolarized $\Delta\Psi_m$ emerged quickly (<1 min) following the onset of ischemia. Interestingly, zones of rapid $\Delta\Psi_m$ depolarization during the early phase of ischemia formed the originating focus of "waves" of $\Delta\Psi_m$ depolarization, which actively propagated across the epicardial surface with a mean velocity of ~20 μm/s (Fig. 3). During the course of ischemia, an expanding zone of $\Delta\Psi_m$ depolarization (red) remained sharply demarcated from the polarized zone ahead of the wavefront producing a very large spatial gradient of $\Delta\Psi_m$. As mentioned previously, such gradients are a key biophysical requirement for metabolic sink, as they may promote conduction block in the local region of depolarized $\Delta\Psi_m$.

7.2 $\Delta\Psi_m$ recovery upon reperfusion

Prompt reperfusion is required for preventing irreversible cell damage and death. Unfortunately, restoration of blood flow, in itself, results in additional cardiac damage, known as reperfusion injury, which results from large bursts of ROS. ROS-mediated oxidative damage is more severe when reperfusion therapy is delayed. Effective strategies to limit or prevent reperfusion injury have proven elusive. Despite an improved understanding of the pathophysiology of this

Fig. 3 Contour maps depicting spatio-temporal changes in the mitochondrial membrane potential during the course of ischemic injury. An organized wave of $\Delta\Psi_m$ depolarization actively propagates across the heart with an average speed of ~20 μm/s. Adapted from ref [28]
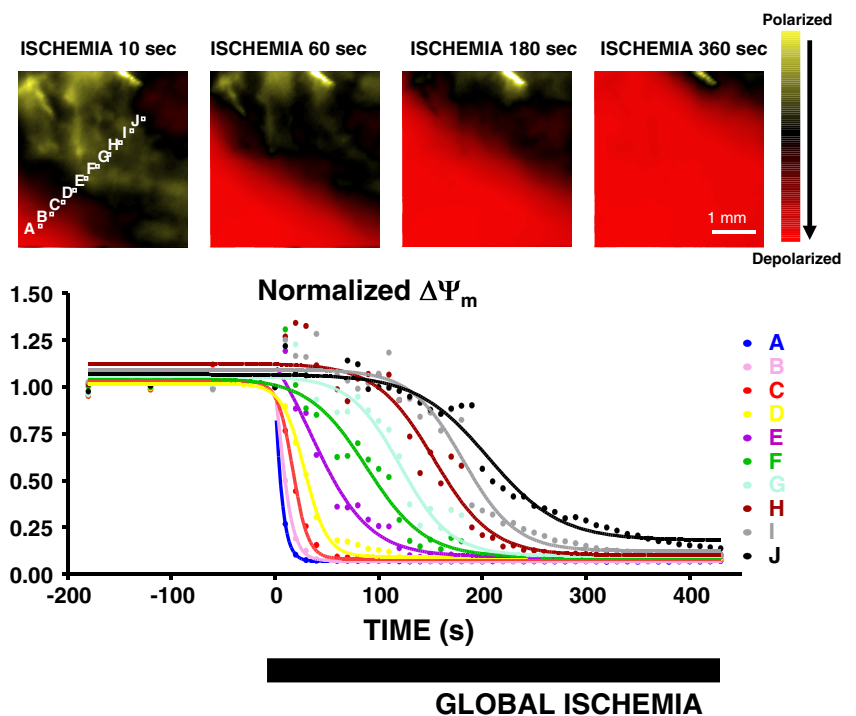
process, the vast majority of clinical trials aimed at preventing reperfusion injury have been quite disappointing. We have demonstrated that the successful recovery of $\Delta\Psi_m$ upon reperfusion is indeed highly dependent on the duration of the preceding ischemic episode (Fig. 4). Interestingly, sustained $\Delta\Psi_m$ recovery was also predictive of post-ischemic functional and electrical recovery (Fig. 4) [28]. These findings reinforce the notion that reperfusion is a highly complex phenomenon which could either reverse or exacerbate ischemia-mediated changes in $\Delta\Psi_m$. In fact, additional $\Delta\Psi_m$ depolarization upon reperfusion following long episodes of ischemia is consistent with ROS-induced damage during this phase [28]. Strategies aimed at promoting rapid recovery of $\Delta\Psi_m$ during the early (first 5 min) phase of reperfusion, potentially by ischemic or pharmacologic post-conditioning strategies, may be an effective strategy for avoiding the genesis of ventricular fibrillation [28]. It is important to note that intracellular calcium overload and calcium-mediated triggers are central features of these arrhythmias. Whether stabilization of $\Delta\Psi_m$ inhibits post-

ischemic arrhythmias through a calcium-dependent mechanism is not known. However, emerging evidence implicates mitochondria as important hubs of calcium buffering and beat-to-beat calcium cycling in the heart. Indeed, the close physical and functional interaction between networks of mitochondria and the sacroplasmic reticulum may be integral to calcium-mediated arrhythmogenesis in various disease states, including I/R injury.

7.3 Role of IMAC in I/R-mediated arrhythmias

Although earlier studies showed that IMAC blockade was effective in abolishing $\Delta\Psi_m$ and cellular AP oscillations, the impact on arrhythmias remained unexplored. We investigated whether protection against mitochondrial depolarization could effectively translate into an anti-arrhythmic benefit in a clinically relevant scenario, such as I/R injury. Indeed, IMAC blockade blunted ischemia-induced APD shortening and the onset of inexcitability in a dose-dependent manner [15]. In


Fig. 4 Reperfusion following short (7.5 min) but not longer (15 min) episodes of global ischemia is associated with full and sustained recovery of $\Delta\Psi_m$. Failure of $\Delta\Psi_m$ recovery during early reperfusion (first 5 min) is associated with post-ischemic electrical and contractile dysfunction. Representative traces of volume conducted ECG (red) and LV cavity pressure (blue) measured during early reperfusion after 7.5 min (left) and 15 min (right) of ischemia. Adapted from ref [28]
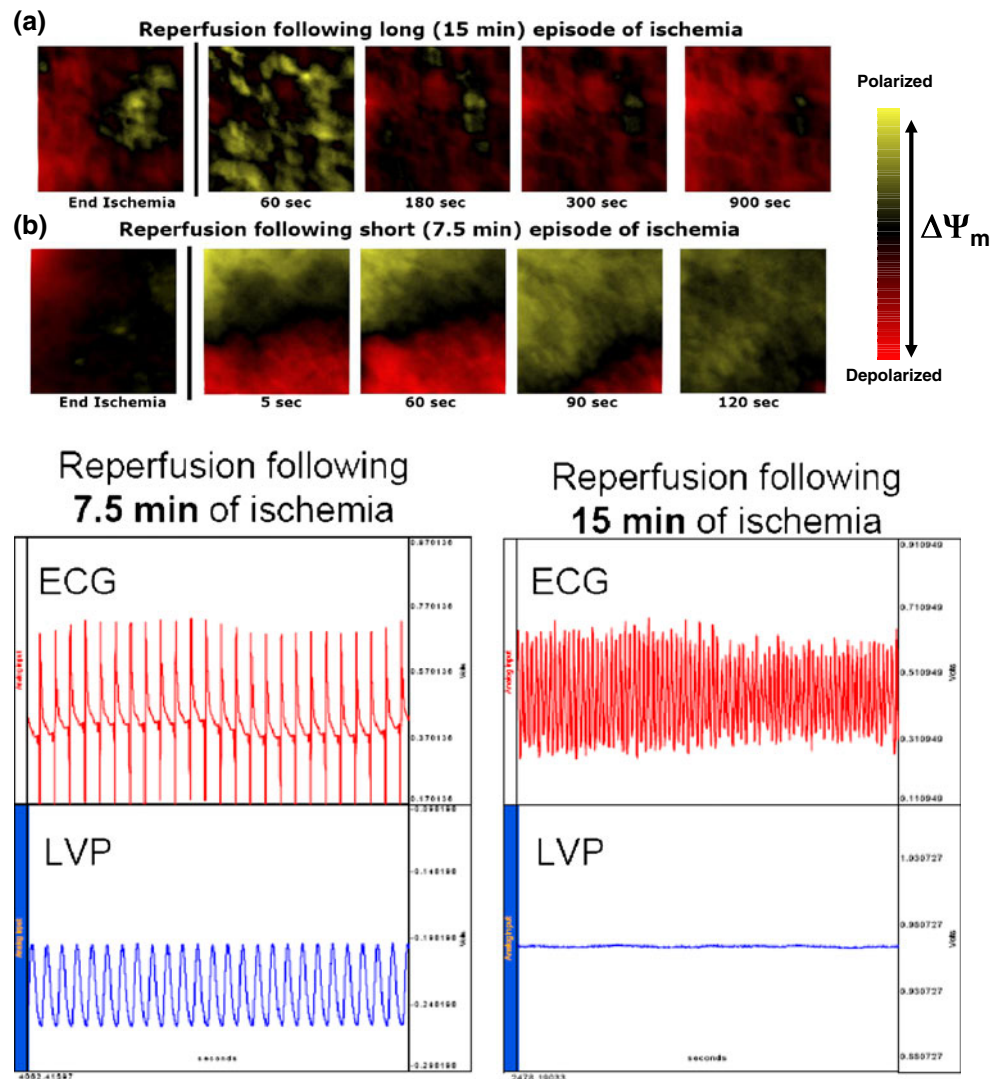
contrast, IMAC activation using agonists of the mBzR led to an accelerated shortening of the AP and an early form of conduction failure during ischemia (Fig. 5) [15]. Specifically, hearts that underwent IMAC activation prior to ischemia exhibited heightened sensitivity to ischemia. Using high-resolution optical AP mapping, we identified discrete areas of conduction block in these hearts during early ischemia that persisted upon reperfusion and likely promoted the formation of reentrant activity underlying post-ischemic VF (Fig. 5). Remarkably, IMAC blockade, which stabilizes $\Delta\Psi_m$ in vitro,

suppressed the formation of these arrhythmias and promoted the rapid recovery of the AP upon reperfusion (Fig. 6). Indeed, these data suggest that mitochondrial depolarization is the primary factor driving sarcK$_{ATP}$ channel activation in ischemia and arrhythmias upon reperfusion. The protective effect of IMAC blockade on post-ischemic electrical function was extended to a rabbit model of I/R injury [25]. This anti-arrhythmic effect was not evident in hearts treated with the mPTP blocker, CsA, reinforcing IMAC as the primary mito-chondrial mediator of acute post-ischemic arrhythmias.

Fig. 5 Metabolic sink/block as a mechanism of conduction failure and arrhythmias. *Top*: Sequential isopotential contour maps that display the level of membrane potential (color coded) across 464 epicardial sites simultaneously. *Red* indicates depolarized membrane potential, and *blue* indicates resting membrane potential. These maps demonstrate the sequential spread of activation across the epicardium of a representative guinea pig heart at 11 min of ischemia. This heart was treated with an IMAC activator (agonist of the mitochondrial benzodiazepine receptor) prior to ischemia. IMAC activation promotes early conduction block as the wavefront fails to propagate across the entire mapping field (i.e., metabolic sink/block). *Bottom*: Representative AP traces recorded at 11 min of ischemia (*left*) and 10 min of reperfusion (*right*) indicating presence of conduction block (*left*) and arrhythmias with electrical silence at the same sites of conduction block (*right*). Adapted from ref [15]
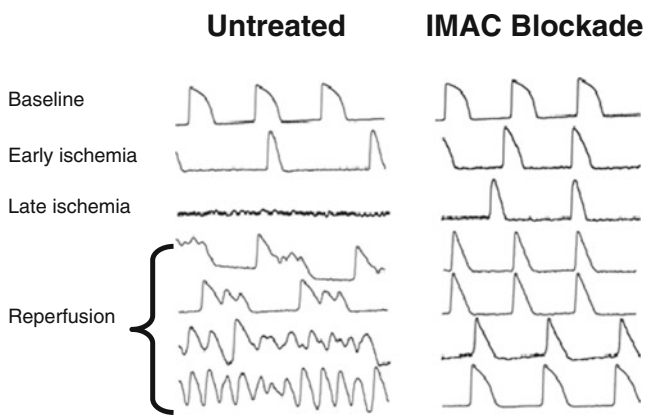
Untreated   **IMAC Blockade**

Baseline

Early ischemia

Late ischemia
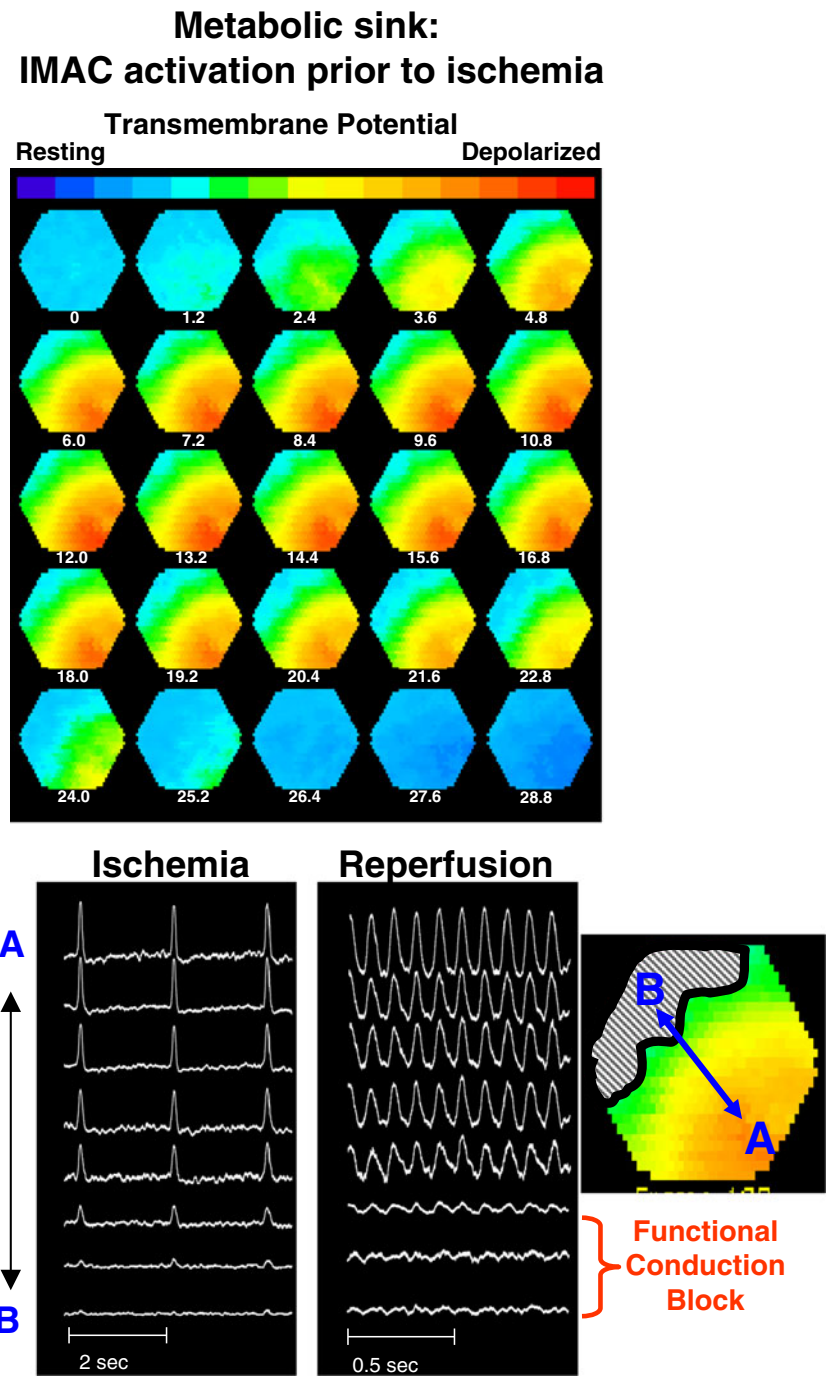
Reperfusion

Fig. 6 Representative action potential traces recorded at baseline, during ischemia, and early reperfusion in an untreated guinea pig heart (*left*) and a heart that underwent IMAC blockade using the mitochondrial benzodiazepine receptor antagonist, 4′-Cl-DZP (60 μM). IMAC blockade protected against post-ischemic VF and promoted the rapid recovery of the action potential following reperfusion. Adapted from ref [15]

8 Mitochondrial dysfunction in LVH

Mitochondria are central to the pathophysiology of the hypertrophied heart. For example, a marked shift in substrate utilization (free fatty acids to glucose) for energy production has been documented in various animal models and humans with hypertrophy [34]. In addition, major structural deformities in cardiac mitochondria, including increased volume and disrupted network architecture, occur in hypertrophy. These fundamental changes contribute significantly to the progression of cardiac dysfunction and remodeling by disrupting the balance between pro- and antioxidant pathways in favor of OS and associated mitochondrial dysfunction (Fig. 1). We recently investigated the spatio-temporal properties of $\Delta\Psi_m$ in the hypertrophied rat heart and found that $\Delta\Psi_m$ depolarization was paradoxically prevented during challenge with short episodes of ischemia [35]. Protection against $\Delta\Psi_m$ depolarization in the rat model of ascending aortic banding-induced LVH was not, however, associated with protection against post-ischemic arrhythmias [35]. Our findings regarding altered $\Delta\Psi_m$ properties are consistent with earlier reports. Notably, Nagendran and colleagues [36] reported an increase of $\Delta\Psi_m$ in phenylephrine-treated rat neonatal cardiomyocytes, adult rat hearts from animals with monocrotaline-induced pulmonary hypertension, and human tissue samples from patients with right ventricular hypertrophy undergoing cardiac surgery [36]. In contrast, Sharma et al. [37] demonstrated that hypertrophied guinea pig myocytes are associated with a basal reduction of $\Delta\Psi_m$. It is worth noting that in the study by Sharma et al. [37], animals were examined at an advanced stage of the disease, which reflected the transition point from cardiac hypertrophy to decompensated heart failure. As such, a phase switch (high

to low) in $\Delta\Psi_m$ may dictate the transition from compensated to decompensated heart failure, an intriguing notion that requires direct investigation. Nagendran and colleagues [36] linked the increase in $\Delta\Psi_m$ to changes in pyruvate metabolism, whereas Sharma et al. [37] emphasized the activation of apoptotic pathways through ROS. Interestingly, mPTP opening during calcium-mediated stress is more likely to occur in diseased compared to normal hearts [38], suggesting mPTP blockade as an important therapeutic target during late stage remodeling. Indeed, chronic sodium–hydrogen exchange inhibition with cariporide was found to confer major benefits in the setting of advanced LVH, in part via mPTP inhibition [39].

Altered patterns of $\Delta\Psi_m$ polarity in hypertrophy could indeed represent an adaptive mechanism, with beneficial or deleterious EP consequences. Both elevations and depressions of $\Delta\Psi_m$ can lead to increased ROS production [40]. An increase in $\Delta\Psi_m$, for example, may provide a protective mechanism against apoptosis, a process that is central to the transition from compensated hypertrophy to end-stage heart failure. On the other hand, increased $\Delta\Psi_m$ may also interfere with mitochondrial substrate transport and respiration and thus underlie some of the adverse metabolic remodeling observed in hypertrophy, including a major shift in substrate utilization from free fatty acids to glucose. In an elegant study, Chen et al. [41] investigated the differential effects of inhibiting glycolysis versus oxidative phosphorylation on $\Delta\Psi_m$ depolarization and arrhythmia propensity in embryonic mouse hearts. Inhibition of oxidative phosphorylation but not glycolysis caused major $\Delta\Psi_m$ depolarization. However, both strategies led to comparable slowing of heart rate, shortening of APD, blunting of the intracellular calcium transients, and promotion of arrhythmias [41]. Of note, the developing myocardium and the hypertrophied heart are both highly dependent on glycolysis for energy production when compared to normal adult myocardium. This potentially explains the paradoxical resistance of the hypertrophied heart to $\Delta\Psi_m$ collapse but not to arrhythmias, which we observed in the rat aortic banding model of pressure overload LVH.

Multiple changes in mitochondrial ion fluxes may alter bioenergetics by increasing $\Delta\Psi_m$ in the setting of cardiac hypertrophy. Importantly, reduced activity of mitochondrial calcium channels, as demonstrated in human heart failure, would lower the influx of calcium into the mitochondrial matrix [42]. Moreover, the development of hypertrophy is associated with marked changes in the expression of UCP, which are known to regulate $\Delta\Psi_m$ and ROS generation. In fact, the finding of $\Delta\Psi_m$ hyperpolarization is consistent with UCP down-regulation which has been observed in animal models and humans with hypertrophy [20]. The potential arrhythmic consequences of these mitochondrial pathways remain largely unexplored. However, in a recent study, Ozcan et al. reported increased vulnerability to I/R-mediated arrhythmias of a transgenic mouse model lacking UCP3

[43]. This heightened susceptibility was linked to deficient myocardial bioenergetics and increased ROS generation [43]. Although, mild pharmacological uncoupling of mitochondria with low-dose FCCP has been shown to induce a cardioprotective effect through ROS-dependent signaling [44], recent reports have linked mitochondrial uncoupling to ventricular fibrillation through activation of sarcK$_{ATP}$ channels in rats [45] and increased interventricular EP heterogeneity in rabbits [46]. Clearly, a systematic understanding of how altered mitochondrial bioenergetics in response to mitochondrial uncoupling deregulates EP function and predisposes to arrhythmias will require further investigation.

Finally, we observed a spatially heterogeneous $\Delta\Psi_m$ substrate in hypertrophied compared to normal hearts during I/R injury. Since $\Delta\Psi_m$ instability leads to APD instability through cyclical activation of sarcK$_{ATP}$ channels, it is conceivable that increased $\Delta\Psi_m$ heterogeneity in the intact hypertrophied heart may underlie APD heterogeneity. This, in turn, is expected to enhance dispersion of repolarization, forming a suitable substrate for reentrant arrhythmias (Fig. 1). Direct evidence for this concept, however, remains lacking in the setting of LVH. Nonetheless, IMAC blockade, which suppresses metabolic sink, has shown multiple important benefits in isoprenaline-induced hypertrophy, including decreased heart-to-body weight ratio and apoptosis (65, 70). As such, IMAC blockade may also confer anti-arrhythmic benefits indirectly by initiating disease regression pathways.

9 Mitochondrial targets for anti-arrhythmic intervention

Mitochondria are highly dynamic organelles that form intricate networks across the cardiomyocyte. These networks strongly influence cardiac function through ROS signaling and amplification. While ROS production is ordinarily countered by sophisticated antioxidant defense systems, conditions leading to OS can unleash a pathological mitochondrial response termed RIRR, which scales across the mitochondrial network to destabilize $\Delta\Psi_m$ and EP function (Fig. 1). Metabolic sinks driven by $\Delta\Psi_m$ depolarization may predispose to arrhythmias by shortening the effective refractory period and slowing myocardial conduction in the area of the sink, thereby, shortening the excitation wavelength [16]. Moreover, presence of heterogeneous metabolic sinks is expected to promote heterogeneous action potential repolarization across the tissue. Finally, having a discrete region or dispersed loci of metabolic sinks may predispose to arrhythmias either by forming unidirectional conduction block or causing heterogeneous conduction, respectively [16].

In summary, mitochondria are at the epicenter of pathological signaling cascades associated with a broad range of cardiac disorders (Fig. 1). A comprehensive understanding of mechanisms by which altered mitochondrial and metabolic pathways disrupt ion channel and EP function will help identify new and powerful strategies for combating arrhythmias through upstream mechanisms, such as RIRR, rather than downstream effectors or bystanders of these diseases. Development of selective pharmacological agents that target critical elements of these pathways, including key mitochondrial ion channels, will likely impact our ability to suppress commonly encountered arrhythmias.

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