Published in final edited form as:
Pharmacol Ther. 2011 September ; 131(3): 287–294. doi:10.1016/j.pharmthera.2011.04.005.

Mitochondria are sources of metabolic sink and arrhythmias

Fadi G. Akar, PhD[1,2,*] and **Brian O'Rourke, PhD**[2]

[1] Cardiovascular Institute, Mount Sinai School of Medicine, New York, NY USA

[2] Division of Cardiology, Johns Hopkins University, Baltimore, MD, USA

Abstract

Mitochondria have long been recognized for their central role in energy transduction and apoptosis. More recently, extensive work in multiple laboratories around the world has significantly extended the role of cardiac mitochondria from relatively static arbitrators of cell death and survival pathways to highly dynamic organelles that form interactive functional networks across cardiomyocytes. These coupled networks were shown to strongly affect cardiomyoyte responses to oxidative stress by modulating cell signaling pathways that strongly impact physiological properties. Of particular importance is the role of mitochondria in modulating key electrophysiological and calcium cycling properties in cardiomyocytes, either directly through activation of a myriad of mitochondrial ion channels or indirectly by affecting cell signaling cascades, ATP levels, and the over-all redox state of the cardiomyocyte. This important recognition has ushered a renewed interest in understanding, at a more fundamental level, the exact role that cardiac metabolism, in general and mitochondria, in particular, play in both health and disease. In this article, we provide an overview of recent advances in our growing understanding of the fundamental role that cardiac mitochondria play in the genesis of lethal arrhythmias.

Keywords

Ischemia-reperfusion injury; mitochondria; reactive oxygen species. Arrhythmias

INTRODUCTION

Mitochondria are well recognized for their importance in energy production and apoptosis (Gustafsson & Gottlieb, 2008). They generate ATP through oxidative phosphorylation, driven by electron transport across the electron transport chain. In addition, mitochondria generate reactive oxygen species (ROS) which have diverse cell signaling functions (Droge, 2002; Becker, 2004). A key metric of mitochondrial function is the mitochondrial membrane potential ($\Delta\psi_m$) which forms the proton-motive force used to produce ATP (O'Rourke, 2007). In normal hearts, $\Delta\psi_m$ is tightly regulated such that the production of ATP is maintained within a physiological range that matches energy production to demand (O'Rourke, 2007). This limits ROS generation and oxidative stress. In ischemia-reperfusion

Address Correspondence to: Fadi G. Akar, PhD, Cardiovascular Institute, Mount Sinai School of Medicine, One Gustave L. Levy Place, Atran-berg 511, New York, NY 10029, Tel: 212-241-9251, Fax: 212-241-4080, fadi.akar@mssm.edu.

injury, $\Delta\psi_m$ is disrupted, altering over-all energy and redox balance within cardiac myocytes (Honda *et al.*, 2005).

Seminal work has extended the role of mitochondria from static arbitrators of cell death and survival pathways to highly dynamic organelles that form interactive networks across cardiomyocytes. These coupled networks strongly affect cardiomyocyte responses to oxidative stress by modulating cell signaling pathways. This important recognition has ushered a renewed interest in understanding, at a more fundamental level, the exact role that cardiac mitochondria play in both health and disease (Michelakis, 2008). In this review, we focus on the role of mitochondrial dysfunction in promoting cardiac electrophysiological abnormalities at the cellular level and malignant arrhythmias at the tissue-network level.

Mitochondrial Criticality and Metabolic Oscillations

Zorov *et al* (Zorov *et al.*, 2000; Zorov *et al.*, 2006) advanced the notion of ROS-induced ROS-release (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 oxidative stress. 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 (Zorov *et al.*, 2006).

Distinct modes of RIRR have been postulated based on their dependence on various mitochondrial ion channels (Yang *et al.*). Specifically, Zorov *et al* (Zorov et al., 2000; Zorov et al., 2006) 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 (Zorov *et al.*, 2000). On the other hand, studies by Aon *et al*. (Aon *et al.*, 2003) provided strong evidence in support of the inner membrane anion channel (IMAC) as a mediator of RIRR and associated electrophysiological and 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 and not the mPTP. Once a threshold level of ROS was exceeded across a critical mass of the mitochondrial network (ie mitochondrial criticality), sustained $\Delta\psi_m$ oscillations were initiated (Aon *et al.*, 2006; Aon *et al.*, 2009). Similar $\Delta\psi_m$ oscillations are also generated in isolated myocytes subjected to oxidative stress via substrate deprivation (Romashko *et al.*, 1998), ATP depletion (Ryu *et al.*, 2005), diamide (Aon *et al.*, 2007), and respiratory inhibition (Ryu *et al.*, 2005). Recent evidence using two-photon microscopy confirmed these cellular data as reversible collapses in $\Delta\psi_m$ were observed in intact hearts exposed to global ischemia/reperfusion or diamide administration (Slodzinski *et al.*, 2008). As will be discussed next, these mitochondrial oscillations can result in cellular electrophysiological oscillations via cyclical activation of sarcolemmal K-ATP ($sarcK_{ATP}$) channels providing compelling evidence of a mechanistic link between mitochondrial dynamics and cellular electrical dysfunction. In what follows, we describe the downstream ionic mediator of electrical inexcitability caused by mitochondrial dysfunction, followed by a discussion of key upstream mechanisms that regulate arrhythmias, including mitochondrial ion channels and the redox state of the cardiomyocyte.

Down-stream mediator of metabolic stress: Role of sarcolemmal K_{ATP} channels

$SarcK_{ATP}$ channels link membrane excitability to metabolism (Nichols, 2006). They are regulated by intracellular nucleotides, membrane phospholipids, protein kinases and phosphatases (Nichols, 2006). $SarcK_{ATP}$ channel activation can precede cellular ATP depletion because the open probability of these channels is increased when cofactors like ADP, pH and Mg^{2+} begin to rise. $SarcK_{ATP}$ channels activate rapidly when mitochondria

uncouple because the drop in $\Delta\psi_m$ due to increased proton leak causes the reversal of the ATP synthase, thus consuming cytoplasmic ATP and decreasing the phosphorylation potential. Tight coupling between the mitochondrial energy state and sarcK_{ATP} channel activation is facilitated by the high energy phosphoryl transfer reactions of the cytoplasm (Sasaki *et al.*, 2001).

Due to their abundance in the plasma membrane, the opening of sarcK_{ATP} channels causes rapid action potential shortening, loss of intracellular K^+, and reduction in myocyte excitability (Billman, 2008). In fact, increased K^+ conductance through sarcK_{ATP} channels can effectively lock the resting membrane potential close to the equilibrium potential for K^+ (Kleber, 1983). Indeed, sarcK_{ATP} channel activation accounts for most of the action potential shortening during ischemia, as evidenced by the ability of K_{ATP} channel blockers (ie, glibenclamide) to prevent the decrease in action potential duration during early ischemia (Akar *et al.*, 2005).

The dynamic relationship between sarcK_{ATP} channel activation and the metabolic status of the cardiomyocyte was first observed by O'Rourke and colleagues (O'Rourke *et al.*, 1994; O'Rourke *et al.*, 1995). Following metabolic stress either by substrate deprivation or increased ADP levels, sarcK_{ATP} currents were activated in phase with NADH fluctuations. In these experiments, sustained $\Delta\psi_m$ oscillations occurred in phase with cellular electrophysiological (namely action potential) oscillations that were driven by 'out-of-phase' sarcK_{ATP} current activation (Aon *et al.*, 2003).

While sarcK_{ATP} channel activation is thought to protect the viability of ischemic tissue by limiting calcium cycling and force generation during periods of reduced energy supply, increased potassium conductance through these channels predisposes to electrical dysfunction and arrhythmias (Billman, 1994; Billman *et al.*, 1998; Billman, 2008). The pro-arrhythmic potential of sarcK_{ATP} channel activation during ischemia-reperfusion could be attributed to increased dispersion of repolarization and shortening of the effective refractory period, and therefore the cardiac wavelength, at a time when calcium mediated triggers are known to arise. Moreover, the opening of sarcK_{ATP} channels creates a current sink 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 that we previously termed 'metabolic sink' (Akar *et al.*, 2005).

This pro-arrhythmic potential of sarcK_{ATP} channel activation has been confirmed in multiple studies. Preventing sarcK_{ATP} channel activation by pharmacological blockade of the channel decreased the incidence of ventricular arrhythmias in rat (Vajda *et al.*, 2007), rabbit (Fischbach *et al.*, 2004), pig (Wirth *et al.*, 1999), dog (Billman *et al.*, 1998), and man (Cacciapuoti *et al.*, 1991; Lomuscio *et al.*, 1994; Aronson *et al.*, 2003). On the other hand, 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. In order to understand the factors driving the opening of sarcK_{ATP} channels during metabolic stress, an overview of key mitochondrial ion channels and bioenergetic properties are discussed below.

Mitochondrial ion channels as root causes of mitochondrial dysfunction and arrhythmias

The mitochondrial membrane is a highly resistive structure that maintains a large voltage gradient and proton-motive force, required for electron transport and ATP production (Brown *et al.*, 2010). Nonetheless, a rich diversity of ion channels and transporters has been discovered in the inner and outer membranes of mitochondria. Of note to arrhythmia mechanisms are various ion channels (Figure 1) that modulate $\Delta\psi_m$ and also promote apoptosis (mPTP), cellular inexcitability (IMAC), cardioprotection (mitoKATP), and

mitochondrial calcium influx (MCU). The interested reader is referred to excellent reviews that exclusively cover mitochondrial ion channel targets in a more comprehensive manner (Peixoto *et al.*).

Inner Membrane Anion Channel

Anion flux across the inner mitochondrial membrane was observed in early studies in which anion movement was shown to regulate mitochondrial volume (Azzi & Azzone, 1966, 1967; Brierley, 1970). Since then, the existence of IMAC has been confirmed in multiple studies demonstrating its importance in anion efflux from energized mitochondria (Garlid & Beavis, 1986; Beavis, 1992). Although the exact structure and molecular identity of IMAC remain elusive, the tight regulation of this channel by benzodiazepine compounds (Beavis, 1989) suggests a strong association between a partially anion selective pore-forming subunit in the inner membrane and a peripheral benzodiazepine receptor in the outer membrane.

The importance of IMAC in modulating $\Delta\psi_m$ was first noted when several distinct IMAC ligands were shown to prevent pathological $\Delta\psi_m$ oscillations in isolated cardiac myocytes (Aon *et al.*, 2003). Importantly, blocking $\Delta\psi_m$ oscillations by targeting the IMAC also inhibited action potential oscillations and prevented myocyte inexcitability (Aon *et al.*, 2003). This provided indirect evidence that targeting the IMAC may be an effective strategy for preventing arrhythmias, at least at the cellular level.

Indeed, IMAC blockade successfully prevented post-ischemic arrhythmias in intact myocardium (Akar *et al.*, 2005; Brown *et al.*, 2008b; Brown *et al.*, 2010). Optical mapping of the epicardial surface of guinea pig hearts revealed that IMAC blockade decreased ischemia-induced action potential shortening and markedly suppressed the incidence of ventricular tachycardia/fibrillation during the early onset of reperfusion (Akar *et al.*, 2005). Cardioprotection mediated by IMAC blockade was also observed in isolated rabbit hearts and accompanied by improved left ventricular function (Brown *et al.*, 2008b). Of notable clinical interest, reperfusion arrhythmias in both studies were also prevented when the IMAC blocker was delivered as a bolus injection at the onset of reperfusion (Akar *et al.*, 2005; Brown *et al.*, 2008b).

Mitochondrial Permeability Transition Pore

The role of the mitochondrial permeability transition pore (mPTP) in ischemia/reperfusion injury has received considerable attention (Halestrap *et al.*, 2004; Murphy & Steenbergen, 2008; Halestrap, 2009; Halestrap & Pasdois, 2009). It is clear that the opening of the mPTP plays a significant role in the generation of necrotic and apoptotic cell death, both of which are involved in the etiology of myocardial infarction (McCully *et al.*, 2004). Administration of cyclosporin-A or sanglifehrin-A, both blockers of the mPTP, attenuates myocardial infarction (Weinbrenner *et al.*, 1998; Minners *et al.*, 2000; Hausenloy *et al.*, 2002; Argaud *et al.*, 2004), left ventricular dysfunction (Griffiths & Halestrap, 1993; Clarke *et al.*, 2002; Hausenloy *et al.*, 2004; Oka *et al.*, 2008a), cardiomyocyte death (Nazareth *et al.*, 1991; Duchen *et al.*, 1993; Kim *et al.*, 2006), and ischemia-reperfusion injury (Di Lisa *et al.*, 2001; Oka *et al.*, 2008b). The translation of these findings was recently supported in a clinical study, in which administration of cyclosporin-A immediately prior to percutaneous coronary intervention decreased the extent of short-term injury in a small clinical trial (Piot *et al.*, 2008).

While the role of the mPTP in cell death is well established, its involvement in the generation of arrhythmias remains controversial. While some studies showed moderate protection against arrhythmias, other studies confirmed a lack of protection in rat (Dow *et al.*, 2009), guinea pig (Akar *et al.*, 2005), and rabbit (Brown *et al.*, 2008b) hearts. Moreover,

delivery of a cyclosporin-A bolus prior to stenting did not seem to influence the incidence of ventricular fibrillation in humans (Piot *et al.*, 2008). Lack of protection against arrhythmias by mPTP blockade is also supported by mechanistic studies in isolated myocytes that demonstrate that $\Delta\psi_m$ depolarization caused by substrate deprivation or photo-induced oxidation is not prevented by cyclosporin-A (Romashko *et al.*, 1998; Huser & Blatter, 1999; Zorov *et al.*, 2000; Aon *et al.*, 2003). It is very important to note, however, that by protecting against apoptosis and reducing the size of myocardial infarction following an ischemic insult, mPTP blockade may suppress scar related arrhythmias that are associated with healed myocardial infarction. Also, by inhibiting myocyte loss and improving left ventricular function, this strategy may confer an anti-arrhythmic effect through beneficial mechano-electrical feedback or by hindering the progression of adverse electrical remodeling.

MitoK_{ATP} Channel

Evidence for a mitochondrial ATP-sensitive potassium (mitoK_{ATP}) channel was first observed in rat liver mitochondria (Inoue *et al.*, 1991), and later confirmed in heart (Paucek *et al.*, 1992). The opening of mitoK_{ATP} channels may underlie the cardioprotective effects of preconditioning stimuli by partial dissipation of $\Delta\psi_m$, reduction in the driving force for calcium entry into the mitochondrial matrix, inhibition of apoptosis, and overall improvement in cellular respiration ((O'Rourke, 2000; Gross & Peart, 2003).

Numerous studies have examined the role of mitoK_{ATP} channel activation/blockade in altering infarct size (Takashi *et al.*, 1999; O'Rourke, 2004). In general, mitoK_{ATP} channel blockade with 5-hydroxydecanoate (5-HD) abolished the ability of the cardioprotective stimulus to reduce infarct size (Takashi *et al.*, 1999). While these studies have yielded important mechanistic insights, it is noteworthy that mitoK_{ATP} channel opening also fails to evoke a cardioprotective response when repetitive preconditioning stimuli, such as multiple cycles of ischemia/reperfusion (Schwartz *et al.*, 2002) or chronic exercise (Brown *et al.*, 2005) are administered prior to the main insult, confounding the translation of this strategy to clinical use.

Few studies have examined the role of mitoK_{ATP} channels in the genesis of cardiac arrhythmias. A protective role for mitoK_{ATP} channel activation against arrhythmias has been inferred by experiments demonstrating that mitoK_{ATP} channel blockers consistently abolished the anti-arrhythmic phenotype provided by preconditioning stimuli, such as ischemic preconditioning (Vegh & Parratt, 2002; Rajesh *et al.*, 2004), adenosine (Headrick *et al.*, 2003), delta opioid agonists (Fryer *et al.*, 2000; Fischbach *et al.*, 2003), estrogen (Das & Sarkar, 2006), 3-nitropropionic acid (Basgut *et al.*, 2008), nitroglycerin (Baharvand *et al.*, 2009), noradrenaline (Imani *et al.*, 2008), or endothelin receptor agonists (Das *et al.*, 2007). It is important to note, however, that mitoK_{ATP} channel blockade during other preconditioning stimuli; namely, bradykinin (Driamov *et al.*, 2004), low-flow ischemia (Driamov *et al.*, 2004), peroxynitrite (Kiss *et al.*, 2008), and estradiol (Tsai *et al.*, 2002) failed to attenuate the anti-arrhythmic protection of these stimuli.

Studies investigating the efficacy of direct mitoK_{ATP} channel activation on the suppression of post-ischemic arrhythmias have yielded discrepant results (Schwartz *et al.*, 2002; Headrick *et al.*, 2003). One putative explanation for the discordant findings is that various pharmacological agents used to open mitoK_{ATP} channels are confounded by non-specific action. In fact, the non-specificity of mitoK_{ATP} channel openers (such as diazoxide) and blockers (such as 5-HD) has received considerable attention in recent years (Hanley *et al.*, 2003; Suzuki *et al.*, 2003; O'Rourke, 2004; Brown *et al.*, 2005; Hanley *et al.*, 2005). Moreover, mitoK_{ATP} channel activity is largely dependent on complex signaling cascades,

including phosphorylation by protein kinase C (Ohnuma *et al.*, 2002), which may be differentially altered in various studies.

While the preconditioning literature provides interesting mechanistic insights regarding anti-arrhythmic strategies administered before index ischemia, the clinical relevance of these strategies should be put into question. To the clinician, arrhythmia suppression must often be attempted after, not before, the onset of the ischemic insult. Targeting mitoK_{ATP} channels after the onset of metabolic stress seemed promising based on cellular studies, in which the administration of mitoK_{ATP} channel openers effectively inhibited ongoing $\Delta\psi_m$ oscillations that were evoked by halting respiration (Ryu *et al.*, 2005). This strategy also improved cellular survival and mitochondrial integrity during cellular reoxygenation (Ozcan *et al.*, 2007). Despite these encouraging cellular findings, post-ischemic administration of mitoK_{ATP} channel openers failed to decrease the incidence of arrhythmias (Das & Sarkar, 2005).

Mitochondrial Calcium Uniporter

Although altered intracellular calcium cycling and cytosolic calcium overload are well established sources of arrhythmia triggers and beat-to-beat repolarization abnormalities (Wilson *et al.*, 2006), the role of mitochondrial calcium fluxes in the generation of arrhythmias remains unclear. Mitochondrial calcium homeostasis is achieved by balanced calcium influx into the matrix via the mitochondrial calcium uniporter (MCU) and efflux out of the matrix through the mitochondrial sodium–calcium exchanger. MCU blockade with ruthenium compounds has shown some promise in suppressing the incidence of arrhythmias. Specifically, pre-ischemic administration of both ruthenium red and Ru360 decreased the incidence of ventricular fibrillation upon reperfusion in rats (Garcia-Rivas Gde *et al.*, 2006). Moreover, both compounds converted ongoing ventricular fibrillation to ventricular tachycardia when administered after the onset of arrhythmias, although neither compound led to sinus rhythm (Kawahara *et al.*, 2003).

Mechanisms by which MCU blockade protects against arrhythmias are not well understood but may involve a decrease in the open channel probability of the mPTP by maintaining relatively low matrix calcium concentrations (Garcia-Rivas Gde *et al.*, 2006). While this is largely expected to confer an anti-apoptotic effect, it seems unlikely to play a major role in arrhythmogenesis since blockers of the mPTP have not been particularly effective in preventing arrhythmias, as discussed previously. Indeed, these findings are supported by cellular experiments in which the reversible collapse in $\Delta\psi_m$ induced during RIRR was not prevented by either ruthenium red (Romashko *et al.*, 1998) or Ru360 (Zorov *et al.*, 2000).

The exact role of the mitochondrial calcium uniporter in arrhythmogenesis remains unclear because of major confounding effects of the ruthenium compounds on intracellular calcium fluxes (Griffiths, 2000). For example, Ruthenium red blocks calcium entry through L-type calcium channels (Vassilev *et al.*, 1987) and release from the sarcoplasmic reticulum (Gupta *et al.*, 1989), suggesting that the anti-arrhythmic efficacy of this compound may be related to its prevention of intracellular calcium overload and not to its primary mitochondrial target (Griffiths & Rutter, 2009). Ru360 appears to be more specific for the MCU, but whole heart experiments are confounded by permeability issues, with some investigators showing successful drug entry into myocytes (Kawahara *et al.*, 2003) and others arguing against it (Robert *et al.*, 2001; Bell *et al.*, 2006). Consistent with their ability to reduce cytosolic calcium transients, both ruthenium compounds are potent negative inotropes at concentrations that protect against arrhythmias (Gupta *et al.*, 1988; Kimura *et al.*, 2005), an undesirable side effect when the overall purpose of administering the compound is to improve cardiac function. Future research using novel compounds that lack these

pleiotropic/permeability issues will provide better insights into the role of the MCU in post-ischemic arrhythmias.

Anti-oxidant depletion as a mechanism of mitochondrial dysfunction and arrhythmias

Oxidative stress in cardiomyocytes is caused by either increased ROS production and/or reduced scavenging capacity. In fact, myocardial Glutatione (GSH), a main anti-oxidant defense system in myocytes, is a key regulator of RIRR and mitochondrial stability. Interestingly, depletion of the intracellular antioxidant GSH pool with diamide effectively triggers $\Delta\psi_m$ oscillations that are similar in nature to those generated by photo-induced oxidation of the myocyte (Aon *et al.*, 2007). These observations were extended to the level of the whole heart, in which diamide treatment of *ex vivo* perfused hearts resulted in heterogeneous ROS production, $\Delta\psi_m$ depolarization (Slodzinski *et al.*, 2004) and ventricular fibrillation (Brown *et al.*, 2008a). Interestingly, reduced-to-oxidized glutathione ratio (GSH/GSSG) in whole heart homogenates following diamide administration was similar to that in isolated cells undergoing RIRR and $\Delta\psi_m$ oscillations (Aon *et al.*, 2007). These findings are corroborated by human data, where low GSH/GSSG ratios were observed in human heart samples from patients with heart failure (Damy *et al.*, 2009) and type 2 diabetes (Anderson *et al.*, 2009), both important risk factors for cardiac arrhythmias and sudden death. Consistent with this notion, administration of N-acetylcycsteine significantly decreased the incidence of cardiac arrhythmias in patients following cardiac surgery (Ozaydin *et al.*, 2008). While promising, N-acetylcycsteine itself is confounded by limited bioavailability (Holdiness, 1991) and anaphylactoid-like reactions (Holdiness, 1991). This clearly highlights the need for alternative compounds that can more effectively and safely restore GSH levels.

Finally, the redox state of the cardiomyocyte can also modulate its excitability properties through mitochondria-independent mechanisms. For example, increased oxidation has been shown to directly activate sarcK_{ATP} channels (Tokube *et al.*, 1996), alter the inactivation kinetics of L-type calcium channels, decrease sodium current density (Liu *et al.*, 2010), increase ryanodine receptor calcium 'leak' (Belevych *et al.*, 2009), and modulate the activation state of mitochondrial inner membrane ion channels. Attempts to improve the redox status of the cardiomyocyte by scavenging ROS with superoxide dismutase mimetics (Konya *et al.*, 1992) or mitochondria-targeted anti-oxidant peptides (Cho *et al.*, 2007) were successful in decreasing the incidence of arrhythmias. Future experiments that optimize effective delivery of ROS-scavenging agents to mitochondria have clear potential in abrogating electrical abnormalities caused by metabolic dysfunction.

Spatio-temporal dynamics of mitochondrial function across the intact heart

As mentioned above, $\Delta\psi_m$ depolarization is triggered by opening of mitochondrial ion channels under conditions of oxidative stress (Weiss *et al.*, 2003; O'Rourke, 2007; Brown *et al.*, 2010). Specifically, during metabolic insults, increased mitochondrial ROS production from complex III of the electron transport chain triggers the opening of IMAC and/or mPTP (Weiss *et al.*, 2003). This results in ROS release from mitochondria and $\Delta\psi_m$ depolarization. In isolated cardiomyocytes, ROS diffusion within the cytosol triggers further ROS release from neighboring mitochondria, initiating a feedback cycle of RIRR and $\Delta\psi_m$ depolarization (Zhou *et al.*,; Zorov *et al.*, 2000; Aon *et al.*, 2003; Brady *et al.*, 2004).

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 remained 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 (Weiss *et al.*, 2006). We recently

found that the metabolic substrate of the heart during the early onset of ischemia is spatially and temporally heterogeneous (Lyon *et al.*, 2010b). These spatio-temporal heterogeneities in mitochondrial function may ultimately dictate myocardial excitability and contribute to the formation of zones of conduction block by heterogeneous activation of surface K_{ATP} channels, as we had previously speculated (Akar *et al.*, 2005).

$\Delta\psi_m$ depolarization

A semi-quantitative approach of optical $\Delta\psi_m$ imaging in the *ex vivo* perfused heart allowed the identification of waves of $\Delta\psi_m$ depolarization that actively propagate across the myocardium with a mean velocity of ~20μm/sec (Figure 2), several orders of magnitude slower than myocardial action potential propagation (Lyon *et al.*, 2010a). We further elucidated complex spatio-temporal metabolic instabilities that preceded and accompanied the formation of these organized waves (Figure 2). Furthermore, we identified at the tissue level the presence of $\Delta\psi_m$ ripples prior to mitochondrial collapse during ischemia. These data suggested patterns of wave behavior spreading across the myocardium ahead of the main wave of $\Delta\psi_m$ depolarization, with propagation reflecting the direct interaction between adjacent cells within the intact ischemic tissue. Although we did not directly image ROS levels, it is conceivable that ROS diffusion at the interface between depolarized (acting as ROS sources) and polarized (ROS sinks) regions can drive the propagation of $\Delta\psi_m$ collapse, in a manner that extends the notion of RIRR from a subcellular to a multi-cellular phenomenon. The amplification and propagation of $\Delta\psi_m$ depolarization across the electrically coupled syncytium may present novel opportunities to limit injury by potentially targeting areas of early $\Delta\psi_m$ collapse that form the origin of the organized propagating wavefront of mitochondrial dysfunction.

The importance of $\Delta\psi_m$ kinetics at the tissue level was also highlighted in a recent study in which cardiac arrhythmias induced by GSH oxidation were effectively inhibited by preventing $\Delta\psi_m$ depolarization using IMAC blockade (Brown *et al.*, 2010). Paradoxically, we also recently found that $\Delta\psi_m$ depolarization was completely prevented in hypertrophied hearts that were challenged with short episodes of ischemia (Jin *et al.*, 2010). Protection against $\Delta\psi_m$ depolarization in this rat model of ascending aortic banding was not, however, associated with protection against arrhythmias (Jin *et al.*, 2010).

Finally, in embryonic mouse hearts, Chen et al (Chen *et al.*, 2007) elegantly investigated the differential effects of inhibiting glycolysis versus oxidative phosphorylation on $\Delta\psi_m$ depolarization and arrhythmia propensity. While inhibition of oxidative phosphorylation but not glycolysis caused a major depolarization in $\Delta\psi_m$, both strategies led to comparable slowing of heart rate, shortening of the action potential duration, blunting of the intracellular calcium transients, and promotion of arrhythmias (Chen *et al.*, 2007). Of note is the fact that the developing myocardium is more dependent on glycolysis than is the adult heart.

$\Delta\psi_m$ Recovery

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 (Bolli *et al.*, 1989). 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 process, the vast majority of clinical trials aimed at preventing reperfusion injury have been quite disappointing. We recently demonstrated that the successful recovery of $\Delta\psi_m$ upon reperfusion is indeed highly dependent on the duration of the preceding ischemic episode. Despite a comparable degree of $\Delta\psi_m$ depolarization following 7.5 and 15 minutes of global no-flow ischemia in the rat,

reperfusion led to recovery of $\Delta\psi_m$ only following the short (7.5 min) but not longer episodes of ischemia (Lyon *et al.*, 2010b). Interestingly, sustained $\Delta\psi_m$ recovery was also predictive of post-ischemic functional and electrical recovery (Lyon *et al.*, 2010b). 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 (Lyon *et al.*, 2010b). Strategies aimed at promoting rapid recovery of $\Delta\psi_m$ during the early (first 5 minutes) phase of reperfusion, potentially by ischemic or pharmacologic post-conditioning strategies, may be an effective strategy for avoiding the genesis of ventricular fibrillation (Lyon *et al.*, 2010b).

Metabolic sinks and reperfusion arrhythmias

Spatio-temporal heterogeneities in mitochondrial function may be associated with local changes in sarcK_{ATP} current density which could potentially create areas of depressed excitability to form conduction block through a mechanism we termed "metabolic sink" (Akar *et al.*, 2005). The presence of metabolic sinks may promote the genesis of arrhythmias by shortening the effective refractory period and slowing myocardial conduction in the area of the sink; thereby, shortening the excitation wavelength. 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. In support of the concept of metabolic sink, IMAC activation using agonists of the mitochondrial benzodiazepine receptor led to an accelerated shortening of the action potential and an early form of conduction failure during ischemia. In contrast, IMAC blockade delayed action potential shortening and the onset of inexcitability (Akar *et al.*, 2005). In this guinea pig model, sustained ventricular tachyarrhythmias were readily generated upon reperfusion in ~90% of hearts (Akar *et al.*, 2005). Remarkably, IMAC blockade, which stabilizes $\Delta\psi_m$ *in vitro*, markedly suppressed the formation of these arrhythmias. Indeed, these data suggest that mitochondrial depolarization is the primary factor driving K_{ATP} channel activation in ischemia and arrhythmias upon reperfusion. The protective effect of IMAC blockade on electrical and contractile post-ischemic function was further demonstrated in a rabbit model of ischemia reperfusion injury (Brown *et al.*, 2008b). This anti-arrhythmic effect was not evident in hearts treated with the mPTP blocker, cyclosporine A, reinforcing IMAC as the primary mitochondrial mediator of post-ischemic arrhythmias. This concept of metabolic sinks is strengthened by our $\Delta\psi_m$ imaging studies, which revealed complex spatio-temporal dynamics of $\Delta\psi_m$ properties that were closely related to post-ischemic electrical and contractile recovery (Lyon *et al.*, 2010a). Finally, the dependence of electrical dysfunction on $\Delta\psi_m$ was recently argued in hearts that did not undergo ischemia-reperfusion injury, but rather, were challenged with diamide-induced glutathione oxidation. Again, IMAC blockade was effective in preventing both $\Delta\psi_m$ depolarization and arrhythmias in this model of metabolic stress (Brown *et al.*, 2010).

Mitochondria as therapeutic targets

Cardiac mitochondria form a compact three dimensional lattice structure that is tightly packed between myofilaments and surrounding t-tubules. This spatial organization places mitochondria in close proximity to the major sites of energy consumption (myofilaments) and excitation-contraction coupling (diads). By being the major source of ROS production, mitochondria can intricately alter the activity of multiple ion channel, Ca^{2+} handling and contractile proteins. Moreover, the generation of metabolic intermediates within mitochondria provides the reducing equivalents required to maintain the negative redox

potential of cellular antioxidant pathways. As such, mitochondria clearly represent an attractive target for altering myocyte function, including electrophysiological properties.

Uncovering mechanisms by which mitochondrial dysfunction predisposes to arrhythmias will allow us to design novel strategies. Targeting root causes (ie mitochondria) rather than downstream consequences (cell surface membrane transporters, calcium cycling proteins, etc) is expected to be advantageous as mitochondria represent a main hub of myocyte function that controls energetics, cell signaling, calcium handling and electrical function.

The development of effective therapeutic strategies targeting the mitochondrial network is currently hampered by a lack of solid molecular information regarding the identity of key mitochondrial ion channels and transporters. For example, none of the proteins involved in mitochondrial Ca^{2+} homeostasis have thus far been completely resolved. Pharmacological studies point us towards promising targets such as the IMAC, mPTP, mitoK_{ATP}, and MCU, but actual mitochondrial structures and macromolecular complexes that mediate changes in $\Delta\psi_m$ remain a subject of intensive debate and active investigation. Indeed, this field of mitochondrial biology is ripe for discovery as powerful proteomic and genomic tools become more readily available. Meanwhile, integrative multi-scale investigation, involving complementary *in vivo*, *ex vivo*, *in vitro*, and *in silico* approaches is essential for understanding how metabolic failure at the level of the organelle can scale to produce arrhythmias in the whole heart.

References

Akar FG, Aon MA, Tomaselli GF, O'Rourke B. The mitochondrial origin of postischemic arrhythmias. The Journal of clinical investigation. 2005; 115:3527–3535. [PubMed: 16284648]

Anderson EJ, Kypson AP, Rodriguez E, Anderson CA, Lehr EJ, Neufer PD. Substrate-specific derangements in mitochondrial metabolism and redox balance in the atrium of the type 2 diabetic human heart. Journal of the American College of Cardiology. 2009; 54:1891–1898. [PubMed: 19892241]

Aon MA, Cortassa S, Akar FG, Brown DA, Zhou L, O'Rourke B. From mitochondrial dynamics to arrhythmias. Int J Biochem Cell Biol. 2009; 41:1940–1948. [PubMed: 19703656]

Aon MA, Cortassa S, Akar FG, O'Rourke B. Mitochondrial criticality: a new concept at the turning point of life or death. Biochimica et biophysica acta. 2006; 1762:232–240. [PubMed: 16242921]

Aon MA, Cortassa S, Maack C, O'Rourke B. Sequential opening of mitochondrial ion channels as a function of glutathione redox thiol status. The Journal of biological chemistry. 2007; 282:21889–21900. [PubMed: 17540766]

Aon MA, Cortassa S, Marban E, O'Rourke B. Synchronized whole cell oscillations in mitochondrial metabolism triggered by a local release of reactive oxygen species in cardiac myocytes. The Journal of biological chemistry. 2003; 278:44735–44744. [PubMed: 12930841]

Argaud L, Gateau-Roesch O, Chalabreysse L, Gomez L, Loufouat J, Thivolet-Bejui F, Robert D, Ovize M. Preconditioning delays Ca2+-induced mitochondrial permeability transition. Cardiovascular research. 2004; 61:115–122. [PubMed: 14732208]

Aronson D, Mittleman MA, Burger AJ. Effects of sulfonylurea hypoglycemic agents and adenosine triphosphate dependent potassium channel antagonists on ventricular arrhythmias in patients with decompensated heart failure. Pacing Clin Electrophysiol. 2003; 26:1254–1261. [PubMed: 12765455]

Azzi A, Azzone GF. Metabolism-dependent mitochondrial shrinkage coupled to ion movement. Biochimica et biophysica acta. 1966; 120:466–468. [PubMed: 5966550]

Azzi A, Azzone GF. Swelling and shrinkage phenomena in liver mitochondria. VI. Metabolism-independent swelling coupled to ion movement. Biochimica et biophysica acta. 1967; 131:468–478. [PubMed: 4962528]

Baharvand B, Dehaj ME, Rasoulian B, Namdari M, Shikhani Y, Kiani AA. Delayed anti-arrhythmic effect of nitroglycerin in anesthetized rats: involvement of CGRP, PKC and mK ATP channels. International journal of cardiology. 2009; 135:187–192. [PubMed: 18584896]

Basgut B, Aypar E, Basgut E, Akin KO, Kilic N, Cakici I. The mechanism of the late preconditioning effect of 3-nitropropionic acid. Archives of pharmacal research. 2008; 31:1257–1263. [PubMed: 18958415]

Beavis AD. On the inhibition of the mitochondrial inner membrane anion uniporter by cationic amphiphiles and other drugs. The Journal of biological chemistry. 1989; 264:1508–1515. [PubMed: 2492277]

Beavis AD. Properties of the inner membrane anion channel in intact mitochondria. Journal of bioenergetics and biomembranes. 1992; 24:77–90. [PubMed: 1380509]

Becker LB. New concepts in reactive oxygen species and cardiovascular reperfusion physiology. Cardiovascular research. 2004; 61:461–470. [PubMed: 14962477]

Belevych AE, Terentyev D, Viatchenko-Karpinski S, Terentyeva R, Sridhar A, Nishijima Y, Wilson LD, Cardounel AJ, Laurita KR, Carnes CA, Billman GE, Gyorke S. Redox modification of ryanodine receptors underlies calcium alternans in a canine model of sudden cardiac death. Cardiovascular research. 2009; 84:387–395. [PubMed: 19617226]

Bell CJ, Bright NA, Rutter GA, Griffiths EJ. ATP regulation in adult rat cardiomyocytes: time-resolved decoding of rapid mitochondrial calcium spiking imaged with targeted photoproteins. The Journal of biological chemistry. 2006; 281:28058–28067. [PubMed: 16882672]

Billman GE. Role of ATP sensitive potassium channel in extracellular potassium accumulation and cardiac arrhythmias during myocardial ischaemia. Cardiovascular research. 1994; 28:762–769. [PubMed: 7923277]

Billman GE. The cardiac sarcolemmal ATP-sensitive potassium channel as a novel target for anti-arrhythmic therapy. Pharmacology & therapeutics. 2008; 120:54–70. [PubMed: 18708091]

Billman GE, Englert HC, Scholkens BA. HMR 1883, a novel cardioselective inhibitor of the ATP-sensitive potassium channel. Part II: effects on susceptibility to ventricular fibrillation induced by myocardial ischemia in conscious dogs. The Journal of pharmacology and experimental therapeutics. 1998; 286:1465–1473. [PubMed: 9732412]

Bolli R, Jeroudi MO, Patel BS, DuBose CM, Lai EK, Roberts R, McCay PB. Direct evidence that oxygen-derived free radicals contribute to postischemic myocardial dysfunction in the intact dog. Proceedings of the National Academy of Sciences of the United States of America. 1989; 86:4695–4699. [PubMed: 2543984]

Brady NR, Elmore SP, van Beek JJ, Krab K, Courtoy PJ, Hue L, Westerhoff HV. Coordinated behavior of mitochondria in both space and time: a reactive oxygen species-activated wave of mitochondrial depolarization. Biophys J. 2004; 87:2022–2034. [PubMed: 15345578]

Brierley GP. Energy-linked alteration of the permeability of heart mitochondria to chloride and other anions. Biochemistry. 1970; 9:697–707. [PubMed: 5417390]

Brown D, Aon M, Akar F, O'Rourke B. A ligand to the mitochondrial benzodiazepine receptor prevents ventricular arrhythmias and LV dysfunction after ischemia or glutathione depletion. FASEB J. 2008a; 22:747–747.

Brown DA, Aon MA, Akar FG, Liu T, Sorarrain N, O'Rourke B. Effects of 4′-chlorodiazepam on cellular excitation-contraction coupling and ischaemia-reperfusion injury in rabbit heart. Cardiovascular research. 2008b; 79:141–149. [PubMed: 18304929]

Brown DA, Aon MA, Frasier CR, Sloan RC, Maloney AH, Anderson EJ, O'Rourke B. Cardiac arrhythmias induced by glutathione oxidation can be inhibited by preventing mitochondrial depolarization. Journal of molecular and cellular cardiology. 2010; 48:673–679. [PubMed: 19962380]

Brown DA, Chicco AJ, Jew KN, Johnson MS, Lynch JM, Watson PA, Moore RL. Cardioprotection afforded by chronic exercise is mediated by the sarcolemmal, and not the mitochondrial, isoform of the KATP channel in the rat. The Journal of physiology. 2005; 569:913–924. [PubMed: 16223762]

Cacciapuoti F, Spiezia R, Bianchi U, Lama D, D'Avino M, Varricchio M. Effectiveness of glibenclamide on myocardial ischemic ventricular arrhythmias in non-insulin-dependent diabetes mellitus. The American journal of cardiology. 1991; 67:843–847. [PubMed: 1707221]

Chen F, De Diego C, Xie LH, Yang JH, Klitzner TS, Weiss JN. Effects of metabolic inhibition on conduction, Ca transients, and arrhythmia vulnerability in embryonic mouse hearts. American journal of physiology. 2007; 293:H2472–2478. [PubMed: 17660398]

Cho J, Won K, Wu D, Soong Y, Liu S, Szeto HH, Hong MK. Potent mitochondria-targeted peptides reduce myocardial infarction in rats. Coronary artery disease. 2007; 18:215–220. [PubMed: 17429296]

Clarke SJ, McStay GP, Halestrap AP. Sanglifehrin A acts as a potent inhibitor of the mitochondrial permeability transition and reperfusion injury of the heart by binding to cyclophilin-D at a different site from cyclosporin A. The Journal of biological chemistry. 2002; 277:34793–34799. [PubMed: 12095984]

Damy T, Kirsch M, Khouzami L, Caramelle P, Le Corvoisier P, Roudot-Thoraval F, Dubois-Rande JL, Hittinger L, Pavoine C, Pecker F. Glutathione deficiency in cardiac patients is related to the functional status and structural cardiac abnormalities. PloS one. 2009; 4:e4871. [PubMed: 19319187]

Das B, Sarkar C. Is the sarcolemmal or mitochondrial K(ATP) channel activation important in the antiarrhythmic and cardioprotective effects during acute ischemia/reperfusion in the intact anesthetized rabbit model? Life sciences. 2005; 77:1226–1248. [PubMed: 15964023]

Das B, Sarkar C. Similarities between ischemic preconditioning and 17beta-estradiol mediated cardiomyocyte KATP channel activation leading to cardioprotective and antiarrhythmic effects during ischemia/reperfusion in the intact rabbit heart. Journal of cardiovascular pharmacology. 2006; 47:277–286. [PubMed: 16495767]

Das B, Sarkar C, Shankar PR. Pretreatment with sarafotoxin 6c prior to coronary occlusion protects against infarction and arrhythmias via cardiomyocyte mitochondrial K(ATP) channel activation in the intact rabbit heart during ischemia/reperfusion. Cardiovascular drugs and therapy/sponsored by the International Society of Cardiovascular Pharmacotherapy. 2007; 21:243–251. [PubMed: 17520332]

Di Lisa F, Menabo R, Canton M, Barile M, Bernardi P. Opening of the mitochondrial permeability transition pore causes depletion of mitochondrial and cytosolic NAD+ and is a causative event in the death of myocytes in postischemic reperfusion of the heart. The Journal of biological chemistry. 2001; 276:2571–2575. [PubMed: 11073947]

Dow J, Bhandari A, Kloner RA. The mechanism by which ischemic postconditioning reduces reperfusion arrhythmias in rats remains elusive. Journal of cardiovascular pharmacology and therapeutics. 2009; 14:99–103. [PubMed: 19461101]

Driamov S, Bellahcene M, Ziegler A, Barbosa V, Traub D, Butz S, Buser PT, Zaugg CE. Antiarrhythmic effect of ischemic preconditioning during low-flow ischemia. The role of bradykinin and sarcolemmal versus mitochondrial ATP-sensitive K(+) channels. Basic research in cardiology. 2004; 99:299–308. [PubMed: 15221348]

Droge W. Free radicals in the physiological control of cell function. Physiological reviews. 2002; 82:47–95. [PubMed: 11773609]

Duchen MR, McGuinness O, Brown LA, Crompton M. On the involvement of a cyclosporin A sensitive mitochondrial pore in myocardial reperfusion injury. Cardiovascular research. 1993; 27:1790–1794. [PubMed: 8275525]

Fischbach PS, Barrett TD, Reed NJ, Lucchesi BR. SNC-80-induced preconditioning: selective activation of the mitochondrial adenosine triphosphate-gated potassium channel. Journal of cardiovascular pharmacology. 2003; 41:744–750. [PubMed: 12717105]

Fischbach PS, White A, Barrett TD, Lucchesi BR. Risk of ventricular proarrhythmia with selective opening of the myocardial sarcolemmal versus mitochondrial ATP-gated potassium channel. The Journal of pharmacology and experimental therapeutics. 2004; 309:554–559. [PubMed: 14747611]

Fryer RM, Hsu AK, Nagase H, Gross GJ. Opioid-induced cardioprotection against myocardial infarction and arrhythmias: mitochondrial versus sarcolemmal ATP-sensitive potassium channels. The Journal of pharmacology and experimental therapeutics. 2000; 294:451–457. [PubMed: 10900218]

Garcia-Rivas Gde J, Carvajal K, Correa F, Zazueta C. Ru360, a specific mitochondrial calcium uptake inhibitor, improves cardiac post-ischaemic functional recovery in rats in vivo. British journal of pharmacology. 2006; 149:829–837. [PubMed: 17031386]

Garlid KD, Beavis AD. Evidence for the existence of an inner membrane anion channel in mitochondria. Biochimica et biophysica acta. 1986; 853:187–204. [PubMed: 2441746]

Griffiths EJ. Use of ruthenium red as an inhibitor of mitochondrial Ca(2+) uptake in single rat cardiomyocytes. FEBS letters. 2000; 486:257–260. [PubMed: 11119714]

Griffiths EJ, Halestrap AP. Protection by Cyclosporin A of ischemia/reperfusion-induced damage in isolated rat hearts. Journal of molecular and cellular cardiology. 1993; 25:1461–1469. [PubMed: 7512654]

Griffiths EJ, Rutter GA. Mitochondrial calcium as a key regulator of mitochondrial ATP production in mammalian cells. Biochimica et biophysica acta. 2009; 1787:1324–1333. [PubMed: 19366607]

Gross GJ, Peart JN. KATP channels and myocardial preconditioning: an update. American journal of physiology. 2003; 285:H921–930. [PubMed: 12915383]

Gupta MP, Dixon IM, Zhao D, Dhalla NS. Influence of ruthenium red on rat heart subcellular calcium transport. The Canadian journal of cardiology. 1989; 5:55–63. [PubMed: 2465813]

Gupta MP, Innes IR, Dhalla NS. Responses of contractile function to ruthenium red in rat heart. The American journal of physiology. 1988; 255:H1413–1420. [PubMed: 2462366]

Gustafsson AB, Gottlieb RA. Heart mitochondria: gates of life and death. Cardiovascular research. 2008; 77:334–343. [PubMed: 18006487]

Halestrap AP. What is the mitochondrial permeability transition pore? Journal of molecular and cellular cardiology. 2009; 46:821–831. [PubMed: 19265700]

Halestrap AP, Clarke SJ, Javadov SA. Mitochondrial permeability transition pore opening during myocardial reperfusion--a target for cardioprotection. Cardiovascular research. 2004; 61:372–385. [PubMed: 14962470]

Halestrap AP, Pasdois P. The role of the mitochondrial permeability transition pore in heart disease. Biochimica et biophysica acta. 2009; 1787:1402–1415. [PubMed: 19168026]

Hanley PJ, Drose S, Brandt U, Lareau RA, Banerjee AL, Srivastava DK, Banaszak LJ, Barycki JJ, Van Veldhoven PP, Daut J. 5-Hydroxydecanoate is metabolised in mitochondria and creates a rate-limiting bottleneck for beta-oxidation of fatty acids. The Journal of physiology. 2005; 562:307–318. [PubMed: 15513944]

Hanley PJ, Gopalan KV, Lareau RA, Srivastava DK, von Meltzer M, Daut J. Beta-oxidation of 5-hydroxydecanoate, a putative blocker of mitochondrial ATP-sensitive potassium channels. The Journal of physiology. 2003; 547:387–393. [PubMed: 12562916]

Hausenloy DJ, Maddock HL, Baxter GF, Yellon DM. Inhibiting mitochondrial permeability transition pore opening: a new paradigm for myocardial preconditioning? Cardiovascular research. 2002; 55:534–543. [PubMed: 12160950]

Hausenloy DJ, Yellon DM, Mani-Babu S, Duchen MR. Preconditioning protects by inhibiting the mitochondrial permeability transition. American journal of physiology. 2004; 287:H841–849. [PubMed: 15072953]

Headrick JP, Willems L, Ashton KJ, Holmgren K, Peart J, Matherne GP. Ischaemic tolerance in aged mouse myocardium: the role of adenosine and effects of A1 adenosine receptor overexpression. The Journal of physiology. 2003; 549:823–833. [PubMed: 12717009]

Holdiness MR. Clinical pharmacokinetics of N-acetylcysteine. Clinical pharmacokinetics. 1991; 20:123–134. [PubMed: 2029805]

Honda HM, Korge P, Weiss JN. Mitochondria and ischemia/reperfusion injury. Annals of the New York Academy of Sciences. 2005; 1047:248–258. [PubMed: 16093501]

Huser J, Blatter LA. Fluctuations in mitochondrial membrane potential caused by repetitive gating of the permeability transition pore. The Biochemical journal. 1999; 343(Pt 2):311–317. [PubMed: 10510294]

Imani A, Faghihi M, Sadr SS, Keshavarz M, Niaraki SS. Noradrenaline reduces ischemia-induced arrhythmia in anesthetized rats: involvement of alpha1-adrenoceptors and mitochondrial K ATP channels. Journal of cardiovascular electrophysiology. 2008; 19:309–315. [PubMed: 18070031]

Inoue I, Nagase H, Kishi K, Higuti T. ATP-sensitive K+ channel in the mitochondrial inner membrane. Nature. 1991; 352:244–247. [PubMed: 1857420]

Jin H, Nass RD, Joudrey PJ, Lyon AR, Chemaly ER, Rapti K, Akar FG. Altered spatiotemporal dynamics of the mitochondrial membrane potential in the hypertrophied heart. Biophysical journal. 2010; 98:2063–2071. [PubMed: 20483313]

Kawahara K, Takase M, Yamauchi Y. Ruthenium red-induced transition from ventricular fibrillation to tachycardia in isolated rat hearts: possible involvement of changes in mitochondrial calcium uptake. Cardiovasc Pathol. 2003; 12:311–321. [PubMed: 14630297]

Kim JS, Jin Y, Lemasters JJ. Reactive oxygen species, but not Ca2+ overloading, trigger pH- and mitochondrial permeability transition-dependent death of adult rat myocytes after ischemia-reperfusion. American journal of physiology. 2006; 290:H2024–2034. [PubMed: 16399872]

Kimura H, Kawahara K, Yamauchi Y, Miyaki J. On the mechanisms for the conversion of ventricular fibrillation to tachycardia by perfusion with ruthenium red. Journal of electrocardiology. 2005; 38:364–370. [PubMed: 16216614]

Kiss A, Juhasz L, Huliak I, Vegh A. Peroxynitrite decreases arrhythmias induced by ischaemia reperfusion in anaesthetized dogs, without involving mitochondrial KATP channels. British journal of pharmacology. 2008; 155:1015–1024. [PubMed: 18846034]

Kleber AG. Resting membrane potential, extracellular potassium activity, and intracellular sodium activity during acute global ischemia in isolated perfused guinea pig hearts. Circ Res. 1983; 52:442–450. [PubMed: 6831660]

Konya L, Kekesi V, Juhasz-Nagy S, Feher J. The effect of superoxide dismutase in the myocardium during reperfusion in the dog. Free radical biology & medicine. 1992; 13:527–532. [PubMed: 1281132]

Liu M, Liu H, Dudley SC Jr. Reactive oxygen species originating from mitochondria regulate the cardiac sodium channel. Circulation research. 2010; 107:967–974. [PubMed: 20724705]

Lomuscio A, Vergani D, Marano L, Castagnone M, Fiorentini C. Effects of glibenclamide on ventricular fibrillation in non-insulin-dependent diabetics with acute myocardial infarction. Coronary artery disease. 1994; 5:767–771. [PubMed: 7858767]

Lyon AR, Joudrey PJ, Jin D, Nass RD, Aon MA, O'Rourke B, Akar FG. Optical imaging of mitochondrial function uncovers actively propagating waves of mitochondrial membrane potential collapse across intact heart. J Mol Cell Cardiol. 2010a

Lyon AR, Joudrey PJ, Jin D, Nass RD, Aon MA, O'Rourke B, Akar FG. Optical imaging of mitochondrial function uncovers actively propagating waves of mitochondrial membrane potential collapse across intact heart. Journal of molecular and cellular cardiology. 2010b; 49:565–575. [PubMed: 20624394]

McCully JD, Wakiyama H, Hsieh YJ, Jones M, Levitsky S. Differential contribution of necrosis and apoptosis in myocardial ischemia-reperfusion injury. American journal of physiology. 2004; 286:H1923–1935. [PubMed: 14715509]

Michelakis ED. Mitochondrial medicine: a new era in medicine opens new windows and brings new challenges. Circulation. 2008; 117:2431–2434. [PubMed: 18474822]

Minners J, van den Bos EJ, Yellon DM, Schwalb H, Opie LH, Sack MN. Dinitrophenol, cyclosporin A, and trimetazidine modulate preconditioning in the isolated rat heart: support for a mitochondrial role in cardioprotection. Cardiovascular research. 2000; 47:68–73. [PubMed: 10869531]

Murphy E, Steenbergen C. Mechanisms underlying acute protection from cardiac ischemia-reperfusion injury. Physiological reviews. 2008; 88:581–609. [PubMed: 18391174]

Nazareth W, Yafei N, Crompton M. Inhibition of anoxia-induced injury in heart myocytes by cyclosporin A. Journal of molecular and cellular cardiology. 1991; 23:1351–1354. [PubMed: 1811053]

Nichols CG. KATP channels as molecular sensors of cellular metabolism. Nature. 2006; 440:470–476. [PubMed: 16554807]

O'Rourke B. Myocardial K(ATP) channels in preconditioning. Circulation research. 2000; 87:845–855. [PubMed: 11073879]

O'Rourke B. Evidence for mitochondrial K+ channels and their role in cardioprotection. Circulation research. 2004; 94:420–432. [PubMed: 15001541]

O'Rourke B. Mitochondrial ion channels. Annu Rev Physiol. 2007; 69:19–49. [PubMed: 17059356]

O'Rourke B, Ramza BM, Marban E. Oscillations of membrane current and excitability driven by metabolic oscillations in heart cells. Science (New York, NY). 1994; 265:962–966.

O'Rourke B, Ramza BM, Romashko DN, Marban E. Metabolic oscillations in heart cells. Advances in experimental medicine and biology. 1995; 382:165–174. [PubMed: 8540393]

Ohnuma Y, Miura T, Miki T, Tanno M, Kuno A, Tsuchida A, Shimamoto K. Opening of mitochondrial K(ATP) channel occurs downstream of PKC-epsilon activation in the mechanism of preconditioning. American journal of physiology. 2002; 283:H440–447. [PubMed: 12063319]

Oka N, Wang L, Mi W, Caldarone CA. Inhibition of mitochondrial remodeling by cyclosporine A preserves myocardial performance in a neonatal rabbit model of cardioplegic arrest. The Journal of thoracic and cardiovascular surgery. 2008a; 135:585–593. [PubMed: 18329475]

Oka N, Wang L, Mi W, Zhu W, Honjo O, Caldarone CA. Cyclosporine A prevents apoptosis-related mitochondrial dysfunction after neonatal cardioplegic arrest. The Journal of thoracic and cardiovascular surgery. 2008b; 135:123–130. 130 e121–122. [PubMed: 18179928]

Ozaydin M, Peker O, Erdogan D, Kapan S, Turker Y, Varol E, Ozguner F, Dogan A, Ibrisim E. N-acetylcysteine for the prevention of postoperative atrial fibrillation: a prospective, randomized, placebo-controlled pilot study. European heart journal. 2008; 29:625–631. [PubMed: 18263874]

Ozcan C, Terzic A, Bienengraeber M. Effective pharmacotherapy against oxidative injury: alternative utility of an ATP-sensitive potassium channel opener. Journal of cardiovascular pharmacology. 2007; 50:411–418. [PubMed: 18049309]

Paucek P, Mironova G, Mahdi F, Beavis AD, Woldegiorgis G, Garlid KD. Reconstitution and partial purification of the glibenclamide-sensitive, ATP-dependent K+ channel from rat liver and beef heart mitochondria. The Journal of biological chemistry. 1992; 267:26062–26069. [PubMed: 1464617]

Peixoto PM, Ryu SY, Kinnally KW. Mitochondrial ion channels as therapeutic targets. FEBS letters. 584:2142–2152. [PubMed: 20178788]

Piot C, Croisille P, Staat P, Thibault H, Rioufol G, Mewton N, Elbelghiti R, Cung TT, Bonnefoy E, Angoulvant D, Macia C, Raczka F, Sportouch C, Gahide G, Finet G, Andre-Fouet X, Revel D, Kirkorian G, Monassier JP, Derumeaux G, Ovize M. Effect of cyclosporine on reperfusion injury in acute myocardial infarction. The New England journal of medicine. 2008; 359:473–481. [PubMed: 18669426]

Rajesh KG, Sasaguri S, Suzuki R, Xing Y, Maeda H. Ischemic preconditioning prevents reperfusion heart injury in cardiac hypertrophy by activation of mitochondrial KATP channels. International journal of cardiology. 2004; 96:41–49. [PubMed: 15203260]

Robert V, Gurlini P, Tosello V, Nagai T, Miyawaki A, Di Lisa F, Pozzan T. Beat-to-beat oscillations of mitochondrial [Ca2+] in cardiac cells. The EMBO journal. 2001; 20:4998–5007. [PubMed: 11532963]

Romashko DN, Marban E, O'Rourke B. Subcellular metabolic transients and mitochondrial redox waves in heart cells. Proceedings of the National Academy of Sciences of the United States of America. 1998; 95:1618–1623. [PubMed: 9465065]

Ryu SY, Lee SH, Ho WK. Generation of metabolic oscillations by mitoKATP and ATP synthase during simulated ischemia in ventricular myocytes. Journal of molecular and cellular cardiology. 2005; 39:874–881. [PubMed: 16242144]

Sasaki N, Sato T, Marban E, O'Rourke B. ATP consumption by uncoupled mitochondria activates sarcolemmal K(ATP) channels in cardiac myocytes. Am J Physiol Heart Circ Physiol. 2001; 280:H1882–1888. [PubMed: 11247805]

Schwartz LM, Welch TS, Crago MS. Cardioprotection by multiple preconditioning cycles does not require mitochondrial K(ATP) channels in pigs. American journal of physiology. 2002; 283:H1538–1544. [PubMed: 12234807]

Slodzinski MK, Aon MA, O'Rourke B. Intracellular and intercellular mitochondrial membrane potential oscillations in the Langendorff perfused heart. Biophys J. 2004; 86:461a. [PubMed: 14695289]

Slodzinski MK, Aon MA, O'Rourke B. Glutathione oxidation as a trigger of mitochondrial depolarization and oscillation in intact hearts. Journal of molecular and cellular cardiology. 2008; 45:650–660. [PubMed: 18760283]

Suzuki M, Saito T, Sato T, Tamagawa M, Miki T, Seino S, Nakaya H. Cardioprotective effect of diazoxide is mediated by activation of sarcolemmal but not mitochondrial ATP-sensitive potassium channels in mice. Circulation. 2003; 107:682–685. [PubMed: 12578868]

Takashi E, Wang Y, Ashraf M. Activation of mitochondrial K(ATP) channel elicits late preconditioning against myocardial infarction via protein kinase C signaling pathway. Circulation research. 1999; 85:1146–1153. [PubMed: 10590241]

Tokube K, Kiyosue T, Arita M. Openings of cardiac KATP channel by oxygen free radicals produced by xanthine oxidase reaction. The American journal of physiology. 1996; 271:H478–489. [PubMed: 8770087]

Tsai CH, Su SF, Chou TF, Lee TM. Differential effects of sarcolemmal and mitochondrial K(ATP) channels activated by 17 beta-estradiol on reperfusion arrhythmias and infarct sizes in canine hearts. The Journal of pharmacology and experimental therapeutics. 2002; 301:234–240. [PubMed: 11907179]

Vajda S, Baczko I, Lepran I. Selective cardiac plasma-membrane K(ATP) channel inhibition is defibrillatory and improves survival during acute myocardial ischemia and reperfusion. European journal of pharmacology. 2007; 577:115–123. [PubMed: 17904545]

Vassilev PM, Kanazirska MP, Tien HT. Ca2+ channels from brain microsomal membranes reconstituted in patch-clamped bilayers. Biochimica et biophysica acta. 1987; 897:324–330. [PubMed: 2434130]

Vegh A, Parratt JR. The role of mitochondrial K(ATP) channels in antiarrhythmic effects of ischaemic preconditioning in dogs. British journal of pharmacology. 2002; 137:1107–1115. [PubMed: 12429584]

Weinbrenner C, Liu GS, Downey JM, Cohen MV. Cyclosporine A limits myocardial infarct size even when administered after onset of ischemia. Cardiovascular research. 1998; 38:678–684. [PubMed: 9747435]

Weiss JN, Korge P, Honda HM, Ping P. Role of the mitochondrial permeability transition in myocardial disease. Circ Res. 2003; 93:292–301. [PubMed: 12933700]

Weiss JN, Yang L, Qu Z. Systems biology approaches to metabolic and cardiovascular disorders: network perspectives of cardiovascular metabolism. Journal of lipid research. 2006; 47:2355–2366. [PubMed: 16946414]

Wilson LD, Wan X, Rosenbaum DS. Cellular alternans: a mechanism linking calcium cycling proteins to cardiac arrhythmogenesis. Annals of the New York Academy of Sciences. 2006; 1080:216–234. [PubMed: 17132786]

Wirth KJ, Rosenstein B, Uhde J, Englert HC, Busch AE, Scholkens BA. ATP-sensitive potassium channel blocker HMR 1883 reduces mortality and ischemia-associated electrocardiographic changes in pigs with coronary occlusion. The Journal of pharmacology and experimental therapeutics. 1999; 291:474–481. [PubMed: 10525061]

Yang L, Korge P, Weiss JN, Qu Z. Mitochondrial oscillations and waves in cardiac myocytes: insights from computational models. Biophysical journal. 98:1428–1438. [PubMed: 20409461]

Zhou L, Aon MA, Almas T, Cortassa S, Winslow RL, O'Rourke B. A reaction-diffusion model of ROS-induced ROS release in a mitochondrial network. PLoS Comput Biol. 6:e1000657. [PubMed: 20126535]

Zorov DB, Filburn CR, Klotz LO, Zweier JL, Sollott SJ. Reactive oxygen species (ROS)-induced ROS release: a new phenomenon accompanying induction of the mitochondrial permeability transition in cardiac myocytes. The Journal of experimental medicine. 2000; 192:1001–1014. [PubMed: 11015441]

Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial ROS-induced ROS release: an update and review. Biochimica et biophysica acta. 2006; 1757:509–517. [PubMed: 16829228]

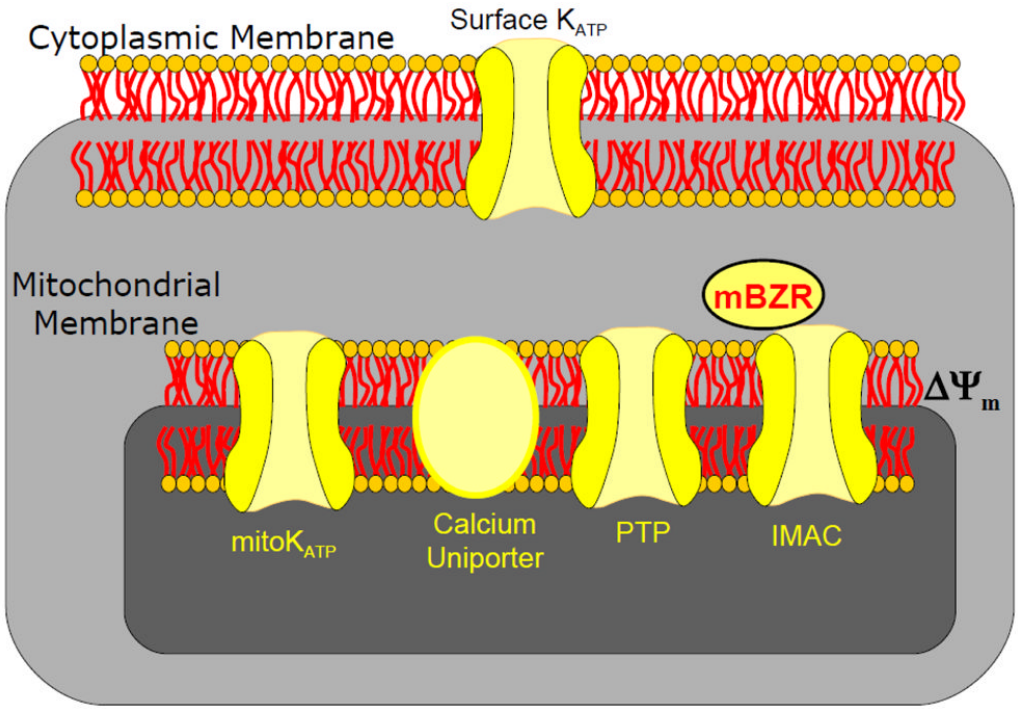

Figure 1.
Schematic of key energy sensitive ion channels that can promote cell survival, death, or arrhythmias.

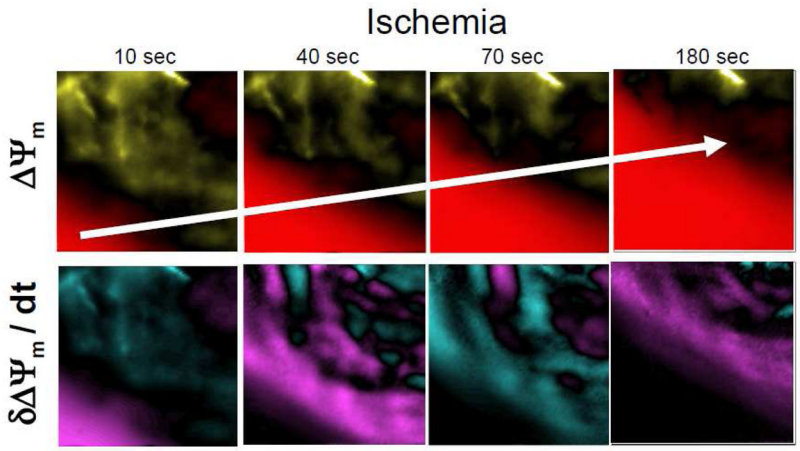

Figure 2. Spatio-temporal fluctuations of $\Delta\psi_m$ during global ischemia (Adapted from Figure 4, Lyon et al. J Mol Cell Cardiol. 2010, PMID: 20624394)

Successive contour maps of normalized $\Delta\psi_m$ (above) and its first derivative (below) acquired at 10, 40, 70, and 180 seconds following the onset of global no-flow ischemia in a representative rat heart. These data illustrate the presence of spatially and temporally discordant kinetics of $\Delta\psi_m$ that exist ahead of the main depolarization wave of $\Delta\psi_m$ collapse, which actively propagates across the heart. Color scale: a) $\Delta\psi_m$ contour maps: baseline (black), depolarization (red), hyperpolarization (yellow); b) $\delta\Delta\psi_m/\delta t$ contour maps: baseline (black), positive slopes (turquoise), negative slopes (purple).