

# Cardiac mitochondria and arrhythmias

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Despite a high prevalence of sudden cardiac death throughout the world, the mechanisms that lead to ventricular arrhythmias are not fully understood. Over the last 20 years, a growing body of evidence indicates that cardiac mitochondria are involved in the genesis of arrhythmia. In this review, we have attempted to describe the role that mitochondria play in altering the heart's electrical function by introducing heterogeneity into the cardiac action potential. Specifically, we have focused on how the energetic status of the mitochondrial network can alter sarcolemmal potassium fluxes through ATP-sensitive potassium channels, creating a 'metabolic sink' for depolarizing wave-fronts and introducing conditions that favour catastrophic arrhythmia. Mechanisms by which mitochondria depolarize under conditions of oxidative stress are characterized, and the contributions of several mitochondrial ion channels to mitochondrial depolarization are presented. The inner membrane anion channel in particular opens upstream of other inner membrane channels during metabolic stress, and may be an effective target to prevent the metabolic oscillations that create action potential lability. Finally, we discuss therapeutic strategies that prevent arrhythmias by preserving mitochondrial membrane potential in the face of oxidative stress, supporting the notion that treatments aimed at cardiac mitochondria have significant potential in attenuating electrical dysfunction in the heart.

**Keywords** Mitochondria • Arrhythmia • Reactive oxygen species • Ischaemia • Reperfusion • Heart • Ion channel • Review • Oscillations • Membrane potential

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## 1. Introduction

Cardiovascular disease is a leading cause of worldwide death in both men and women.<sup>1</sup> Among the manifestations of cardiovascular disease, a significant cause of mortality is sudden cardiac death resulting from malignant ventricular arrhythmia. Despite differences in lifestyle factors across the global population, the frequency of sudden cardiac death is remarkably similar in North America, Europe, and Asia, affecting ~1 of every 1000 people and accounting for as much as one-third of all cardiac deaths in high-risk populations.<sup>2,3</sup> Novel treatments seeking to decrease the incidence of sudden cardiac death clearly have enormous potential for global health.

Investigations into the electrical function of the heart began over 150 years ago when Kölliker and Müller<sup>4</sup> demonstrated that the heart produced electricity that was associated with muscle contraction. Building upon Sydney Ringer's initial discoveries of an ionic basis for heart function,<sup>5,6</sup> significant strides have been made in our understanding of the cellular events that can be modulated to influence the heart's rhythm. In the 1960s, compounds such as amiodarone and lidocaine were first used to treat arrhythmia by inhibiting sarcolemmal ion fluxes, and imaging techniques with increasing resolution are constantly improving our insight into tissue-level events that

lead to arrhythmia. Despite these technical advances in understanding and diagnosing cardiac rhythm disturbances, the underlying mechanistic bases for cardiac arrhythmias are still being elucidated, reflecting a window for therapeutic potential as these sub-cellular pathways responsible for aberrant conduction are illuminated. In this review, we seek to highlight the role that cardiac mitochondria play in influencing myocyte excitability, emphasizing the potential for emergent therapeutic strategies converging on mitochondria to preserve cardiac electrical function. The majority of our focus herein will concentrate on the etiology of ventricular arrhythmias evoked under conditions of oxidative stress. We will highlight potential preventative approaches taken from the animal literature, with pertinent references from human studies included where appropriate.

## 2. Action potential heterogeneity and cardiac arrhythmias

As a syncytium, coordinated electrical propagation throughout the heart is obligatory for adequate function. At the cellular level, each individual myocyte must depolarize and repolarize in a specific manner based on anatomical location. Pathological heterogeneity in

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the cardiac action potential is commonly linked to ventricular arrhythmias, and several sub-cellular factors can contribute to lability in action potential duration. Among the cellular culprits involved in cardiac arrhythmias, ion channels in the sarcolemmal and mitochondrial inner membranes have received considerable attention for their ability to influence action potential duration. Sarcolemmal ion channel mutations leading to prolongation of the action potential (i.e. long QT syndrome), early- or delayed after depolarizations due to activation of calcium channels/exchangers, and altered *trans*-sarcolemmal ion gradients have all been extensively described in their arrhythmogenic role.<sup>7</sup> In this article we will discuss the role that cardiac mitochondria play in influencing cardiomyocyte action potential duration, underscoring therapeutic potential for arrhythmia using mitochondria-targeted approaches.

### 3. Role of sarcolemmal $K_{ATP}$ channels in arrhythmia

Emerging evidence indicates that the mitochondria induce non-physiological spatiotemporal heterogeneity in the cardiac action potential and predispose the heart to re-entrant arrhythmia. The influence of mitochondrial energetic status on the sarcolemmal action potential is mediated in large part by energy-sensing ATP-sensitive potassium channels (sarco $K_{ATP}$ ) in the sarcolemmal membrane. A significant amount of attention has been devoted to the role that sarco $K_{ATP}$  channels may play in inducing action potential heterogeneity, leading to cardiac arrhythmias.<sup>8,9</sup> First discovered in the early 1980s,<sup>10</sup> myocardial sarco $K_{ATP}$  channels are heteromultimers composed of four pore-forming subunits and four accessory subunits, the sulfonylurea receptors, that bind to ATP. Inhibited by intracellular ATP and activated by ADP, Pi, Mg, and/or pH, sarco $K_{ATP}$  channels open under conditions of oxidative stress to produce an inwardly rectifying background current, typically observed within the first 10 min of ischaemia.<sup>11</sup> Sarco $K_{ATP}$  channels are among the most densely populated ion channels in cardiac myocardium,<sup>12</sup> and the opening of even 1% of the total amount of channels in the sarcolemma can significantly shorten the cardiac action potential.<sup>13</sup>

The opening of sarco $K_{ATP}$  channels may be an endogenous protective mechanism of the myocardial tissue, where channel opening signaled by inadequate ATP supply decreases calcium-mediated cardiac energy demand. As the population of sarco $K_{ATP}$  opens, the cardiac action potential shortens and reduces the calcium transient. Since calcium overload can lead to necrotic and apoptotic cell death, sarco $K_{ATP}$  channel opening is believed to be cytoprotective by decreasing the extent contracture by the myofilaments and blunting mitochondrial calcium overload. Several lines of evidence indicate that the expression of functional sarco $K_{ATP}$  channels is vital to cellular survival in the face of oxidative stress. First, increased sarco $K_{ATP}$  protein expression correlated with protection against ischaemia/reperfusion injury in female (vs. male) animals<sup>14–17</sup> or following exercise training.<sup>14,18,19</sup> Second, pharmacological block of the sarco $K_{ATP}$  channel population increased cell death in hearts exposed to ischaemia/reperfusion,<sup>15,18,20</sup> with the block during ischaemia being the critical period leading to increased injury.<sup>15</sup> Third, genetic knockout of the sarco $K_{ATP}$  channel pore-forming subunit led to animals that were severely intolerant to exercise and displayed enhanced sensitivity to calcium overload.<sup>21–23</sup> Taken together, it appears that there is a physiological role for sarco $K_{ATP}$  opening in attenuating cell death during ischaemia.

Consistent with this notion are observations in humans where diabetic patients taking oral sulfonylureas to control type II diabetes were at a higher disposition for cardiac injury following ischaemia.<sup>24</sup>

While the opening of sarco $K_{ATP}$  channels appears to be protective of the viability of ischaemic cardiac myocytes, the consequence of increasing potassium conductance to the whole organ predisposes to electrical dysfunction and in some cases the generation of fatal arrhythmia.<sup>8,25–28</sup> With such a high density of channels in the sarcolemmal membrane, the opening of sarco $K_{ATP}$  channels can significantly shorten the action potential, and if enough channels open, can render cells inexcitable by holding the membrane potential very close to potassium's Nernst equilibrium potential. This creates a tremendous current sink for the propagating depolarization wave, and arrhythmias may be favoured when there are local regions where the open probability of sarco $K_{ATP}$  channels is high (i.e. where the energetic status of the cell has been compromised), a phenomenon our group has previously termed 'metabolic sinks'.<sup>29,30</sup> The presence of metabolic sinks enhances propensity for arrhythmia by influencing the effective refractory period (ERP) of the myocardium, resulting in a shortened excitation wavelength (the product of conduction velocity and refractory period). Pathological heterogeneity in action potential duration increases the 'dispersion of refractoriness' within the tissue, and is known to promote re-entry.<sup>31–33</sup> Sarco $K_{ATP}$  opening abbreviates the action potential duration and shortens ERP. Sarco $K_{ATP}$  channel openers<sup>34–37</sup> and blockers<sup>38</sup> decrease and increase ERP, respectively, and ERP is also prolonged after knockout of sarco $K_{ATP}$  pore-forming subunits.<sup>39</sup> However, other factors may come into play during ischaemia that alter the relationship between action potential duration and ERP. For example, although ischaemia activates sarco $K_{ATP}$  and shortens the action potential, a prolonged ERP may occur due to post-repolarization refractoriness,<sup>40,41</sup> presumably due to alterations in Na channel availability.

An arrhythmogenic role for sarco $K_{ATP}$  has been confirmed in studies using either glibenclamide, which blocks both the mitochondrial and sarcolemmal isoforms of the  $K_{ATP}$  channels, or the sarcolemmal-specific HMR 1833 compounds (or HMR 1098, the sodium salt of HMR 1883). Blocking sarco $K_{ATP}$  channels with HMR1883 decreased the incidence of ventricular arrhythmia in rat,<sup>42</sup> rabbit,<sup>43</sup> pig,<sup>44</sup> and dog.<sup>26</sup> Importantly, the findings from the animal literature are confirmed in clinical studies where sarco $K_{ATP}$  channel blockers reduced the incidence of ventricular fibrillation in humans.<sup>45–47</sup>

While it seems plausible that the prevention of arrhythmias with sarco $K_{ATP}$  blockers is due to direct inhibition of sarco $K_{ATP}$  currents, sarco $K_{ATP}$  blockers could theoretically also indirectly prevent arrhythmias. Specifically, by inhibiting sarco $K_{ATP}$  currents and preventing action potential shortening, the ensuing cellular calcium overload may promote gap junction closure and block re-entrant wave-fronts via cellular uncoupling.<sup>48</sup> In order to understand the factors driving the opening of sarco $K_{ATP}$  channels during metabolic stress, an overview of the underlying bioenergetic events leading to sarco $K_{ATP}$  activation will be presented.

### 4. Metabolic oscillations

The cardiac mitochondrial network produces over 95% cellular ATP, and accounts for ~20–30% of myocardial volume in species ranging from mouse to man.<sup>49</sup> According to the classic chemiosmotic theory as proposed by Mitchell,<sup>50</sup> mitochondria create a proton motive force by pumping protons out of the mitochondrial matrix, and use this

proton electrochemical gradient to liberate the energy needed to phosphorylate ADP to ATP by the  $F_1F_0$ -ATPase. The majority of the proton motive force is comprised of the mitochondrial membrane potential ( $\Delta\Psi_m$ ), with the magnitude of  $\Delta\Psi_m$  being  $\sim 150$  mV in energized mitochondria.<sup>51</sup> Decreases in  $\Delta\Psi_m$  diminish the amount of free energy available to generate ATP, with mitochondria shifting to ATP hydrolysis under pathophysiological conditions when the  $\Delta\Psi_m$  collapses substantially (depicted in *Figure 1A*).

The dynamic relationship between  $K_{ATP}$  current and the metabolic status of heart cells was first observed by O'Rourke and colleagues.<sup>52</sup> Following metabolic stress via substrate deprivation, or in response to increased ADP levels, glibenclamide-sensitive current oscillations were observed in cardiomyocytes. Oscillating  $\text{sarcK}_{ATP}$  currents were observed in phase with NADH fluctuations, and were not influenced by changing cytosolic calcium concentrations. Importantly, the vacillating  $\text{sarcK}_{ATP}$  currents directly influenced cardiac repolarization and introduced significant lability in the length of the action potential waveform.<sup>52</sup> Subsequent studies confirmed the initial observation of oscillatory  $\text{sarcK}_{ATP}$  currents and action potential duration in cardiac myocytes under conditions of metabolic stress.<sup>53,54</sup>

The fluctuations in  $\text{sarcK}_{ATP}$  currents, and consequently action potential duration, are intricately linked to the behavior of the mitochondrion. Collapses in  $\Delta\Psi_m$  have been observed in a number of studies where the myocardium is subjected to oxidative stress, with  $\text{sarcK}_{ATP}$  current increasing in phase with losses of  $\Delta\Psi_m$ .<sup>54</sup> Using cationic lipophilic rhodamine fluorescent probes, several studies have noted reversible collapses in  $\Delta\Psi_m$  in isolated cells subjected to oxidative stress via substrate deprivation,<sup>55</sup> ATP depletion,<sup>53</sup> local generation of reactive oxygen species (ROS),<sup>54</sup> the thiol oxidant diamide,<sup>56</sup> and respiratory inhibition.<sup>53</sup> Recent evidence using two-photon microscopy confirms cellular data as reversible collapses in  $\Delta\Psi_m$  were seen in intact hearts exposed to global ischaemia/reperfusion or diamide.<sup>57</sup>

In addition to nucleotide-dependent activation of  $\text{sarcK}_{ATP}$  currents after loss of  $\Delta\Psi_m$ , the collapse of bioenergetics might also activate  $\text{sarcK}_{ATP}$  currents through mechanical stretch. In this scenario, the loss of mitochondrial function would quickly preclude development of tension and result in paradoxical segment lengthening of the ischaemic ventricular tissue. Given that both ischaemia and stretch activate  $\text{sarcK}_{ATP}$  channels,<sup>58–60</sup> bulging of the myocardium may also contribute to the activation of  $\text{sarcK}_{ATP}$  channels. This mechanism of arrhythmogenesis is supported in studies where preventing dyskinesia reduced extracellular potassium accumulation.<sup>61</sup>

In order to understand the mechanistic basis for collapses in  $\Delta\Psi_m$  that contribute to arrhythmia, an overview of putative mitochondrial ion channels that may be involved will be discussed.

## 5. Role of mitochondrial ion channels in cardiac arrhythmias: inner membrane anion channel

Several distinct energy-dissipating ion channels in the inner membrane have been proposed to be involved in the  $\Delta\Psi_m$  collapse, contributing to the generation of arrhythmia. The first of these channels to be discussed is the inner membrane anion channel (IMAC).

Anion flux across the inner mitochondrial membrane was first observed over 40 years ago,<sup>62–64</sup> with early studies primarily interested in the contribution of anion movement on mitochondrial volume regulation. Since the initial observations, the IMAC has been

characterized in a number of tissues and is believed to play an important role in anion efflux from energized mitochondria (for review, see<sup>65,66</sup>). Although (as with other inner membrane ion channels) the exact structure of the IMAC is not currently known, the sensitivity of the anion channel to regulation by benzodiazepine compounds<sup>67</sup> suggests that the molecular composition consists of an anion channel subunit that associates with a peripheral benzodiazepine receptor in the outer membrane.

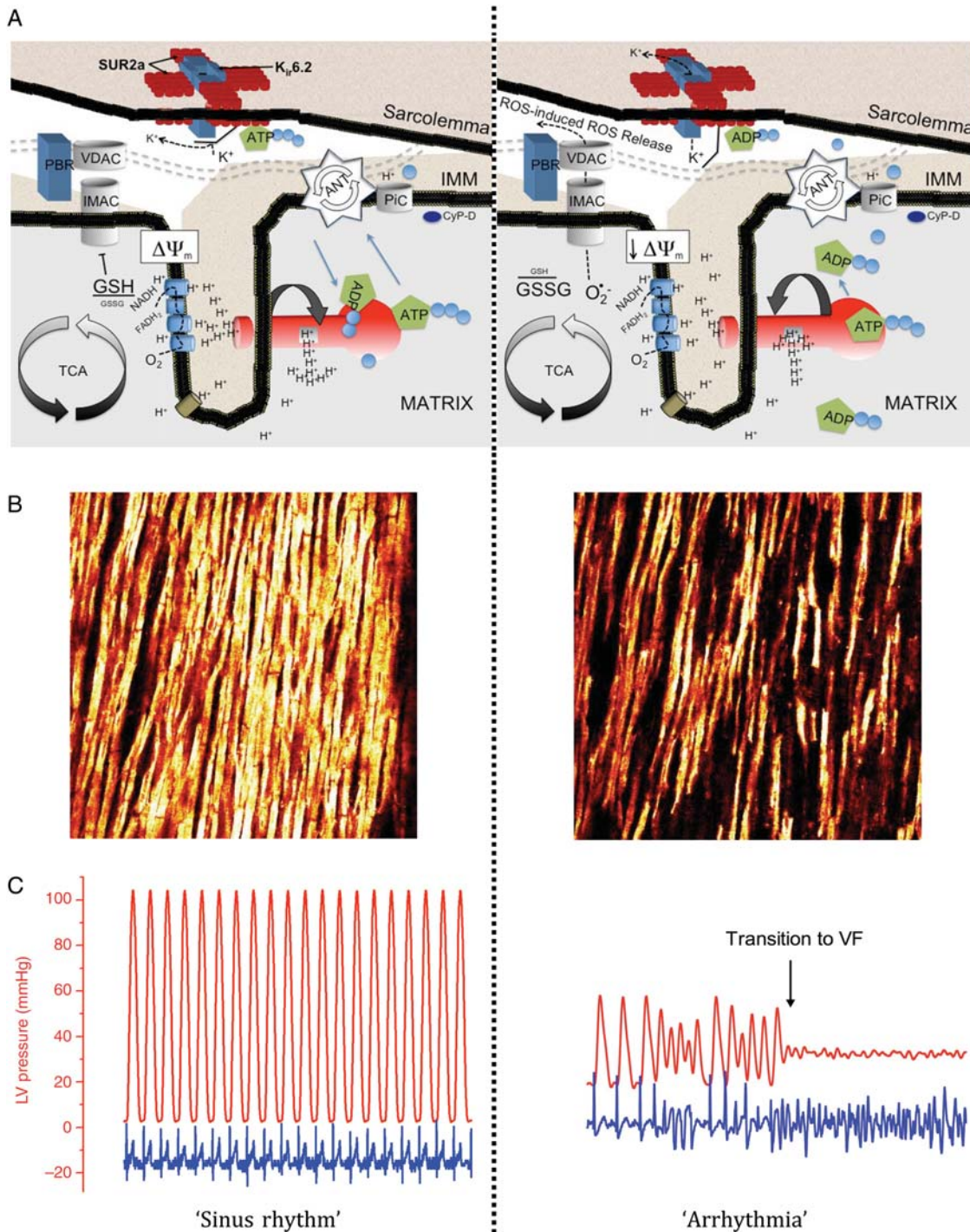
Insights into the factors mediating the collapse in  $\Delta\Psi_m$  have focused on the production of ROS by the mitochondria. ROS-dependent oscillations in  $\Delta\Psi_m$  were first noted by Sollott's group.<sup>68</sup> In their study, Zorov *et al.* noted that local generation of ROS produced by laser flash elicited synchronous collapses in  $\Delta\Psi_m$  that were prevented by a ROS scavenger. There is growing evidence that the collapse in  $\Delta\Psi_m$  may be mediated by superoxide anion, leading to cell-wide depolarizations in the myocardium through a process coined 'ROS-induced ROS release'.<sup>68,69</sup> According to this theory, ROS produced at the level of a single mitochondrion can stimulate superoxide-mediated depolarization of neighbouring mitochondria. This spatiotemporal behavior among the mitochondrial network led our group to conclude that mitochondria are arranged in a percolation matrix.<sup>70</sup> According to empirical data (and corroborated by computer simulations), the increase in ROS under conditions of oxidative stress can reach a critical level, after which cell-wide  $\Delta\Psi_m$  oscillations in the mitochondrial network are observed (deemed 'mitochondrial criticality').<sup>71,72</sup>

The importance of IMAC in influencing the  $\Delta\Psi_m$  was first noted when several distinct ligands to IMAC were found to prevent loss of  $\Delta\Psi_m$  observed in isolated cardiac myocytes. Aon *et al.*<sup>54</sup> used a laser flash to induce a local burst of mitochondrial ROS, which causes cell-wide increases in ROS production and oscillations in  $\Delta\Psi_m$ . The reversible collapses in  $\Delta\Psi_m$  (and the cell-wide ROS accumulation) could be prevented with the addition of PK11195, 4-chlorodiazepam, or DIDS, three distinct compounds that have all been previously shown to block the activity of IMAC.<sup>65,73</sup> Importantly, blocking the reversible collapses in  $\Delta\Psi_m$  by targeting the IMAC stopped the oscillations in action potential duration,<sup>54</sup> providing further cellular evidence that targeting the IMAC may be effective in preventing arrhythmias by stopping ROS-induced ROS release.

A confirmatory role for IMAC involvement in arrhythmia was provided in a series of studies where inhibiting the IMAC prevented arrhythmias in intact mammalian hearts.<sup>29,74,75</sup> Optical mapping of the epicardial surface of guinea pig hearts revealed that blocking IMAC decreased ischaemia-induced action potential shortening and was accompanied by a lack of ventricular tachycardia/fibrillation at the onset of reperfusion.<sup>29</sup> Cardioprotection evoked by blocking the IMAC was also observed in isolated rabbit heart and was accompanied by significantly improved left ventricular developed pressure.<sup>74</sup> Of notable clinical interest, in both studies the reperfusion arrhythmias were prevented when the IMAC was blocked only at the onset of reperfusion (as opposed to pre-treatment).<sup>29,74</sup>

## 6. Mitochondrial permeability transition pore

More attention has been devoted to the activity of the mitochondrial permeability transition pore (PTP) in ischaemia/reperfusion injury than any other mitochondrial inner membrane protein complex.



**Figure 1** Cascade of events where the opening of energy-dissipating anion channels in the mitochondrial inner membrane (IMM) leads to a depolarization of the mitochondrial network, opening of  $\text{sarK}_{\text{ATP}}$  channels, and ultimately transition to arrhythmia in the intact organ. (A) Schematic depiction of the IMM under conditions of normoxia associated with sinus rhythm (left) and during metabolic stress (right). Matrix oxidation is characterized by glutathione oxidation and opening of IMAC, which collapses the  $\Delta\Psi_m$ , leading to ROS-induced ROS release in the mitochondrial network. Structures for IMAC and the PTP are speculative and based on previous reports.<sup>77,166</sup> (B) Two-photon images of  $\Delta\Psi_m$  in an intact guinea pig heart under normoxic (left) and oxidative (right) conditions. (C) Recordings of LV pressure (red trace) and ECG in a guinea pig heart subjected to normoxic and oxidative conditions. The time course for (B) and (C) are similar for both images, indicating that collapse of  $\Delta\Psi_m$  was accompanied by transition to fatal ventricular arrhythmia. Panels (B) and (C) are reprinted from Brown *et al.*<sup>75</sup> with permission from Elsevier.

Extensive characterization of the putative composition and importance of the PTP in ischaemia/reperfusion injury has been put forth, and the reader is referred to several excellent reviews in this area.<sup>76–79</sup> It is clear that the opening of the PTP plays a significant role in the generation of necrotic and apoptotic cell death, both of which are involved in the etiology of myocardial infarction.<sup>80</sup> Administration of cyclosporin-A or sangliferin-A, both blockers of the PTP, attenuate several indices of cardiac I/R injury including myocardial infarction,<sup>81–85</sup> left ventricular dysfunction,<sup>86–89</sup> cardiomyocyte death,<sup>90–92</sup> and mitochondrial dysfunction.<sup>93,94</sup> The translation of these studies was recently supported in human data, where administration of cyclosporin-A immediately prior to percutaneous coronary intervention decreased the extent of short-term injury in a small clinical trial.<sup>95</sup>

While the role of PTP opening in tissue death is clear, there is less evidence that the activity of the PTP influences the generation of cardiac arrhythmia, especially those occurring at the onset of reperfusion. In several experiments using isolated cells, collapses in  $\Delta\Psi_m$  observed after substrate deprivation or laser flash were not prevented by the addition of cyclosporin-A.<sup>54,55,68,96</sup> Using two-photon imaging, blocking the PTP was ineffective at preventing the sustained  $\Delta\Psi_m$  collapse in hearts undergoing global ischaemia.<sup>97</sup> Other investigations confirmed a lack of protection against arrhythmia in rat,<sup>98</sup> guinea pig,<sup>29</sup> and rabbit<sup>74</sup> hearts. Finally, delivery of a cyclosporin-A bolus prior to stenting did not influence the incidence of ventricular fibrillation in human subjects.<sup>95</sup>

## 7. MitoK<sub>ATP</sub> channels

Evidence for a mitochondrial ATP-sensitive potassium (mitoK<sub>ATP</sub>) channel was first observed in rat liver mitochondria,<sup>99</sup> and later confirmed in heart.<sup>100</sup> The opening of mitoK<sub>ATP</sub> channels may be important in mediating protective interventions given before the onset of ischaemia by partially dissipating the  $\Delta\Psi_m$ , reducing the driving force for calcium into the mitochondria, and improving cellular respiration secondary to mild swelling of the matrix (reviewed in<sup>9,101,102</sup>).

Most studies that have examined the cardioprotective effect of mitoK<sub>ATP</sub> opening have examined the role of mitoK<sub>ATP</sub> in mediating reductions in infarct size elicited by a single preconditioning stimulus.<sup>102</sup> In most (but not all) of these studies, blocking the mitoK<sub>ATP</sub> with 5-hydroxydecanoate (5-HD) abolished the reduction in infarct size triggered by the stimulus of interest. While single episodes of preconditioning yield mechanistic insight, it is noteworthy that when repetitive stimuli are administered mitoK<sub>ATP</sub> blockade does not reduce the evoked protection, as evidenced by the lack of effect of 5-HD in abolishing the infarct-sparing effects of repetitive ischaemic preconditioning<sup>103</sup> or chronic exercise.<sup>18</sup>

Fewer studies have examined the activity of mitoK<sub>ATP</sub> channels in cardiac arrhythmia. As with the infarction literature, a role for mitoK<sub>ATP</sub> in protecting against arrhythmia is apparent where mitoK<sub>ATP</sub> blockers abolished the anti-arrhythmic phenotype provided by a preconditioning stimulus such as ischaemic preconditioning,<sup>104,105</sup> adenosine,<sup>106</sup> delta opioid agonists,<sup>107,108</sup> estrogen,<sup>109</sup> 3-nitropropionic acid,<sup>110</sup> nitroglycerin,<sup>111</sup> noradrenaline,<sup>112</sup> or endothelin receptor agonists.<sup>113</sup> Although mitoK<sub>ATP</sub> channels appear to be important in mediating the anti-arrhythmic effects of some preconditioning models, their activity is not attributed to all models of preconditioning. For example, blocking the mitoK<sub>ATP</sub> during preconditioning from

bradykinin,<sup>114</sup> low-flow ischaemia,<sup>114</sup> peroxynitrite,<sup>115</sup> or estradiol<sup>116</sup> did not attenuate the anti-arrhythmic protection.

Protection against arrhythmias via direct activation of mitoK<sub>ATP</sub> channels prior to index ischaemia has yielded opposing results, with some investigators showing protection from arrhythmia<sup>106,117</sup> and others showing no beneficial effect.<sup>43,103</sup> One putative explanation for the discordant findings is that the pharmacological agents used to open mitoK<sub>ATP</sub> were different among these studies (minoxidil, diazoxide, and/or BMS-191095), and some of these compounds are plagued by non-specificity (addressed below).

While the preconditioning literature provides interesting mechanistic insight regarding anti-arrhythmic strategies administered before index ischaemia, the clinical relevance of these strategies must be questioned. To the clinician, attenuation of arrhythmias must often be attempted after the onset of ischaemia. Targeting mitoK<sub>ATP</sub> channels after the onset of metabolic stress seemed promising based on cellular experiments, where administration of mitoK<sub>ATP</sub> blockers stopped oscillations in  $\Delta\Psi_m$  evoked by halting respiration,<sup>53</sup> and mitoK<sub>ATP</sub> opening (with diazoxide) improved cellular survival and mitochondrial integrity during cellular reoxygenation.<sup>118</sup> Despite these encouraging cellular data, post-ischaemic administration of mitoK<sub>ATP</sub> openers does not decrease arrhythmias,<sup>117</sup> and post-conditioning interventions have been shown to be independent of the activity of mitoK<sub>ATP</sub> channels.<sup>98</sup> Indeed, the investigators that observed beneficial effects of diazoxide on isolated cells<sup>118</sup> found that the cytoprotective properties of the drug were independent of mitochondrial potassium flux.<sup>119</sup> The non-specificity of commonly used mitoK<sub>ATP</sub> openers (such as diazoxide) and blockers (such as 5-HD) has received a significant amount of attention in the literature, and several papers have addressed this issue in more detail.<sup>18,102,120–123</sup>

## 8. Mitochondrial calcium uniporter

The role that intracellular calcium concentration plays in the generation of arrhythmia has been extensively characterized.<sup>124,125</sup> Early studies going back almost 50 years indicated that decreasing cytosolic calcium fluxes lowered the incidence of arrhythmia,<sup>126,127</sup> paving the way for Class IV anti-arrhythmic agents that decrease arrhythmias by lowering intracellular calcium.

The role of mitochondrial calcium fluxes in the generation of arrhythmia is much less clear. Mitochondrial calcium homeostasis is believed to involve calcium influx into the matrix via the mitochondrial calcium uniporter (MCU), with the major efflux pathway being the mitochondrial sodium–calcium exchanger.<sup>128</sup> Attempts to decrease arrhythmias by blocking MCU with ruthenium compounds have been somewhat effective but only when given prior to ischaemia. Pre-ischaemic administration of both ruthenium red and Ru360 significantly decreased the incidence of ventricular fibrillation in anesthetized rats,<sup>129</sup> and both ruthenium red and Ru360 effectively converted ventricular fibrillation to ventricular tachycardia (although neither compound led to the reversion of the ECG to sinus rhythm).<sup>130</sup>

Speculation regarding the mechanism whereby MCU protects against arrhythmia involves keeping matrix calcium concentrations low, ultimately leading to decreased open probability of the PTP.<sup>129</sup> While this mechanism is likely involved in influencing the tissue survivability, it seems unlikely to play a prominent role in arrhythmogenesis since blockers of the PTP have not been particularly effective in

preventing arrhythmia (addressed above). These findings are supported by experiments in myocytes, where the reversible collapse in  $\Delta\Psi_m$  induced during ROS-induced ROS release was not prevented by either ruthenium red<sup>55</sup> or Ru360.<sup>68</sup>

At present, it is difficult to draw conclusions about the role of the calcium uniporter in arrhythmogenesis due to the confounding effects of the ruthenium compounds on cellular calcium fluxes.<sup>131</sup> Ruthenium red has been shown to block calcium release from the SR<sup>132–134</sup> and L-type calcium channels,<sup>135</sup> suggesting that the effects of this compound in preventing arrhythmias may be from lowering overall cellular calcium and not by directly acting on the mitochondrion.<sup>136</sup> Ru360 appears to be more specific for the MCU, but whole-heart experiments are confounded by permeability issues, with some investigators showing the Ru360 effectively enters cardiac cells<sup>130</sup> and others indicating that it is not permeable.<sup>137,138</sup> Consistent with their ability to reduce cytosolic calcium transients, both ruthenium compounds are potent negative inotropes at concentrations shown to protect against arrhythmias,<sup>139,140</sup> an undesirable side effect when the overall purpose of administering the compound is to improve cardiac function. Future research with novel compounds that lack these pleiotropic/permeability issues will provide better insight into the role of the MCU in reperfusion arrhythmias.

To date, studies examining mitochondrial calcium fluxes have mostly concentrated on the influx of calcium into the matrix via the MCU. One recent study suggested that pressure-puff-induced intracellular  $\text{Ca}^{2+}$  releases were mediated by the mitochondrial efflux pathway, the mitochondrial sodium–calcium exchanger, which could potentially contribute to cardiac electrical dysfunction.<sup>141</sup>

## 9. Contribution of mitochondrial redox status to collapses in $\Delta\Psi_m$

As addressed above, the redox status of heart cells directly influences the cellular excitability. An oxidative shift in the cellular redox potential can promote action potential heterogeneity by modulating several different ion channels. Increased oxidation has been shown to directly activate sarcK<sub>ATP</sub> channels,<sup>142,143</sup> alter the inactivation kinetics of L-type calcium channels via increased calcium 'leak' from the ryanodine receptor,<sup>144</sup> and influence the state of channels on the mitochondrial inner membrane.

Bursts of ROS are observed within the first few minutes of reperfusion, when the propensity for arrhythmia is extremely high.<sup>145,146</sup> Several experiments have induced ventricular arrhythmias under normoxic conditions with delivery of ROS bursts,<sup>147,148</sup> and attempts to scavenge ROS with superoxide dismutase mimetics<sup>149</sup> or mitochondrial-targeted anti-oxidant peptides<sup>150</sup> were successful in decreasing the incidence of arrhythmia. Future experiments that optimize effective delivery of ROS-scavenging agents to mitochondria clearly have significant potential in abrogating electrical dysfunction.

Among the cellular anti-oxidant defenses, several studies have examined the role of the myocardial glutathione (GSH) pool in arrhythmogenesis. Myocardial GSH is the largest anti-oxidant pool in the heart,<sup>151</sup> with the majority of GSH being the reduced (GSH) vs. the oxidized (GSSG) form in healthy tissues. Commonly observed GSH/GSSG ratios in the mammalian heart are  $\sim 200\text{--}300:1$ ,<sup>56,75</sup> with a 50–70% decrease typically observed under conditions of oxidative stress.<sup>75,152–154</sup> Administration of either GSH or *N*-acetylcysteine

(NAC), a glutathione precursor, has been shown to significantly reduce reperfusion arrhythmias.<sup>155–157</sup>

Increasing evidence supports the notion that myocardial GSH is a key regulator of mitochondrial ROS-induced ROS release. Experiments in isolated cardiac myocytes showed that oscillations in  $\Delta\Psi_m$  could be evoked with the thiol-oxidants diamide<sup>56</sup> or diethylacetate,<sup>68</sup> both of which are known to deplete the GSH pool.<sup>75,158,159</sup> Aon *et al.*<sup>56</sup> altered the GSH/GSSG ratio in permeabilized myocytes and induced oscillations in  $\Delta\Psi_m$  (beginning at a GSH/GSSG ratio of 150:1), with the absolute concentration of GSSG being of primary importance in inducing  $\Delta\Psi_m$  collapses. Consistent with the notion that IMAC opens 'upstream' of the PTP and is a crucial therapeutic target, irreversible collapses in  $\Delta\Psi_m$  indicative of PTP opening were not observed until GSH/GSSG ratios fell below 50:1. In other studies using picochambers to simulate cellular ischaemia/reperfusion in isolated myocytes,  $\Delta\Psi_m$  depolarized during reoxygenation with step-wise increases in the oxygen tension. The depolarizations were mediated by increased ROS, and the addition of exogenous GSH prevented the collapses in  $\Delta\Psi_m$  with increasing oxygen tension.<sup>160</sup>

Subsequent experiments confirmed that GSH oxidation evoked collapses in  $\Delta\Psi_m$  in whole hearts,<sup>57,75</sup> which was accompanied by ventricular tachycardia/fibrillation.<sup>75</sup> Interestingly, the GSH/GSSG ratio in whole-heart homogenates following diamide administration was very similar to ratios in isolated cells that led to mitochondrial criticality.<sup>56</sup> Finally, blocking the IMAC during diamide administration completely prevented the loss of  $\Delta\Psi_m$  and protected guinea pig hearts from arrhythmias<sup>75</sup> (see Figure 1 for mechanistic depiction).

## 10. Implications for GSH depletion and arrhythmias in humans

The findings from animal studies that highlight the beneficial effect of reduced GSH on stabilizing mitochondrial function are corroborated by human data, where low GSH/GSSG ratios were observed in human heart samples from patients in heart failure<sup>161</sup> and with type 2 diabetes,<sup>162</sup> two populations that display high risk for cardiac arrhythmias.<sup>2</sup> Consistent with this notion, administration of the NAC significantly decreased the incidence of cardiac arrhythmia in humans following cardiac surgery.<sup>163</sup> While promising, NAC itself is confounded by low bioavailability<sup>164</sup> and anaphylactoid-like reactions in some patients,<sup>164,165</sup> necessitating alternative compounds that can replenish cardiac GSH but lack the potentially harmful side effects of high NAC doses.

## 11. Conclusions

The cardiac mitochondrial network has emerged as a key target for strategies seeking to decrease arrhythmias. As the 'hubs' for cellular metabolism, preserving the integrity of the mitochondria in the face of metabolic stress will significantly improve almost all aspects of cellular function. Expanding our understanding of the molecular composition of inner membrane ion channels, as well as development of agents that home to mitochondria to diminish ROS overload have enormous potential as treatments to preserve  $\Delta\Psi_m$  and prevent lethal ventricular arrhythmias.

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