Effects of Regional Mitochondrial Depolarization on Electrical Propagation
Implications for Arrhythmogenesis

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Background—Sudden cardiac death often involves arrhythmias triggered by metabolic stress. Loss of mitochondrial function is thought to contribute to the arrhythmogenic substrate, but how mitochondria contribute to uncoordinated electrical activity is poorly understood. It has been proposed that the formation of metabolic current sinks, caused by the nonuniform collapse of mitochondrial inner membrane potential (ΔΨm), contributes to re-entrant arrhythmias because ΔΨm depolarization is tightly coupled to the activation of sarcolemmal ATP-sensitive K+ channels, hastening action potential repolarization and shortening the refractory period.

Methods and Results—Here, we use computational and experimental methods to investigate how ΔΨm instability can induce re-entrant arrhythmias. We develop the first tissue-level model of cardiac electrical propagation incorporating cellular electrophysiology, excitation–contraction coupling, mitochondrial energetics, and reactive oxygen species balance. Simulations show that re-entry and fibrillation can be initiated by regional ΔΨm loss because of the disparity of refractory periods inside and outside the metabolic sink. Computational results are compared with the effects of a metabolic sink generated experimentally by local perfusion of a mitochondrial uncoupler in a monolayer of cardiac myocytes.

Conclusions—The results demonstrate that regional mitochondrial depolarization triggered by oxidative stress activates sarcolemmal ATP-sensitive K+ currents to form a metabolic sink. Consequent shortening of the action potential inside, but not outside, the sink increases the propensity for re-entry. ΔΨm recovery during pacing can lead to novel mechanisms of ectopic activation. The findings highlight the importance of mitochondria as potential therapeutic targets for sudden death associated with cardiovascular disease. (Circ Arrhythm Electrophysiol. 2014;7:143-151.)

Key Words: arrhythmias, cardiac KATP channels mitochondria reactive oxygen species

Whether the index event is acute coronary occlusion or is associated with chronic progressive heart disease, sudden cardiac death usually involves a paroxysmal, unpredictable event that precipitates ventricular arrhythmias. Among potential mechanisms implicated in promoting electrical instability, dispersion of refractoriness is thought to be a major factor in the susceptibility to re-entry and fibrillation. Regional heterogeneity of the effective refractory period of the tissue depends on both differences in the action potential (AP) duration (APD) of the individual cardiomyocytes and the conduction velocity. In nonischemic cardiac tissue, unidirectional block occurs when the repolarization gradient exceeds ≈3.2 ms/mm,1,2 and a similar degree of dispersion has been reported to increase the vulnerability to ventricular tachyarrhythmia in intact hearts.3 Dispersion of repolarization is also a prominent feature of cardiac ischemia and reperfusion,4 concurrent with an increase in the spatiotemporal heterogeneity of mitochondrial inner membrane potential (ΔΨm).5,6 Notably, the timing of ΔΨm depolarization during reperfusion7 and ventricular fibrillation coincide.8

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The mechanisms linking mitochondrial dysfunction to electrical instability in the heart are incompletely understood; however, a large body of evidence indicates that ATP-sensitive K+ (KATP) channels are rapidly activated on energy depleton to cause APD shortening and the concomitant elevation of the ST-segment of the ECG.9 Pharmacological inhibition of sarcolemmal KATP channels blunts APD shortening during ischemia,8 and interestingly, KATP antagonists can also prevent arrhythmias elicited by reperfusion10 or β-adrenergic stress.11 In addition, gain-of-function12 or loss-of-function13 mutations in atrial KATP channel subunits have been associated with arrhythmias in humans.

The cellular events occurring upstream of KATP channel activation, that is, the mechanisms that cause an abrupt decrease

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in energy supply, are crucial in understanding how metabolic stress leads to arrhythmias. In this light, mitochondrial reactive oxygen species (ROS)–induced ROS release (RIRR)\textsuperscript{15} has emerged as a key event that underlies \( \Delta W_m \) depolarization in cardiac cells. RIRR refers to the autocalytic production of a burst of ROS by the mitochondrial electron transport chain when mitochondria are subjected to exogenous sources of ROS or when the antioxidant defenses of the mitochondrion or the cell are compromised.\textsuperscript{16} This phenomenon can be synchronized across the entire mitochondrial network of the cardiomyocyte in a process that depends on local ROS diffusion\textsuperscript{17} and appears as a propagated \( \Delta W_m \) depolarization wave\textsuperscript{17} or as sustained self-organized slow oscillations of \( \Delta W_m \) (period, \( \approx 100 \text{ s} \)).\textsuperscript{18,19} The rapid depolarization of \( \Delta W_m \) transforms the mitochondria into consumers, rather than producers, of ATP,\textsuperscript{20} causing a drop in the cellular ATP/ADP ratio, activating K\textsubscript{ATP} channels, and shortening the APD.

The goal of the present study was to investigate how mitochondrial functional instability alters the dynamics of the electrophysiological substrate to increase the vulnerability to arrhythmias. We used a model of the myocardial syncytium that incorporates a ventricular cardiomyocyte model of excitation–contraction coupling, mitochondrial energetics, and RIRR (ECME-RIRR)\textsuperscript{21} to examine how regional oxidative stress initiates a chain of events that leads to re-entry through the formation of a metabolic current sink. The simulation results are supported by experiments in which a metabolic sink is created in a cardiac cell monolayer. Together, the findings lend credence to the hypothesis that spatiotemporal metabolic instability underlies electrical instability during oxidative stress in the heart.

**Methods**

**Model Development**

The cardiomyocyte model used in this study was the ECME-RIRR\textsuperscript{21} model, which is based on experimental observations of oxidative stress–induced metabolic oscillations in intact guinea pig cardiomyocytes, in which \( \Delta W_m \) depolarization is triggered by an ROS-activated inner membrane anion channel.\textsuperscript{14,15} The general scheme of the ECME-RIRR model is shown in Figure 1 in the Data Supplement. To investigate the effects of heterogeneous mitochondrial energetics on cardiac electrical propagation and ventricular arrhythmias at the tissue level, the ECME-RIRR cardiomyocyte model was incorporated into a 2-dimensional finite element model of ventricular tissue (5x5 cm\textsuperscript{2}). ECME-RIRR model parameters were identical to those in the study by Zhou et al\textsuperscript{17} unless indicated otherwise. Electrical activity in the myocardial sheet was described by the monodomain equation.\textsuperscript{22} No-flux conditions on membrane voltage (V\textsubscript{m}) were implemented at the model boundaries.

**Numeric Aspects**

The monodomain equation (a partial differential equation) was discretized at 200 \( \mu \text{m} \) spatial resolution. Temporal discretization relied on an operator splitting scheme,\textsuperscript{23} whereby a forward Euler method was used to solve the partial differential equation, and a custom-tailored integration technique\textsuperscript{24} was used to solve the ordinary differential equations of the ECME-RIRR model. Because ECME-RIRR contains both fast (eg, Ca\textsuperscript{2+} handling) and slow (eg, mitochondrial tricarboxylic acid cycle) responses, the numeric scheme used different time steps to integrate the partial differential equation and ordinary differential equations. Specifically, the partial differential equation was integrated using a time step of 20 \( \mu \text{s} \), and the set of ordinary differential equations was split into groups of variables that operated at similar time scales so that appropriate time steps could be chosen for each group. This numeric scheme leads to a substantial reduction in execution time.\textsuperscript{22}

**Simulation Protocol**

The model simulation protocol is described in detail in the Data Supplement. Briefly, we first simulated the effect of a metabolic current sink formed by regional mitochondrial depolarization (Figure 1A) on electrical wave propagation in the absence or presence of a single-pulse premature stimulus, S\textsubscript{2}. We then investigated the effect of recovery of \( \Delta W_m \) in the metabolic sink on electrical wave propagation during the repolarization phase of the mitochondrial oscillations in the absence of extrastimuli. The effect of lag time between the electrical stimulus and the recovery of mitochondrial energetics (ES-ME\textsubscript{cm}) on the formation of erratic electrical activity was also analyzed.

**Figure 1. Effect of reactive oxygen species (ROS)–induced regional mitochondrial depolarization on metabolic sink formation and electrical wave propagation in a 2-dimensional (2D) tissue model.**

A. Regional mitochondrial inner membrane potential (\( \Delta W_m \)) depolarization in the central region occurs spontaneously when fractional ROS generation by the electron transport chain is increased (ROS shunt increased from the nominal level of 2% to 14% in the central zone). B. Effects of changing K\textsubscript{ATP} channel density (0/\( \mu \text{m}^2 \), 0.8/\( \mu \text{m}^2 \), 1.8/\( \mu \text{m}^2 \), and 3.8/\( \mu \text{m}^2 \)) throughout the model on the action potential at the center of the metabolic sink (action potentials outside of the sink were not affected). C. Propagation of the electrical wave through the metabolic sink at 90 ms after the S\textsubscript{1} stimulus (1 Hz stimulus applied at lower left corner). D. Sodium channel availability (\( \text{[Na]} \); inactivation gate parameter) with different K\textsubscript{ATP} channel densities. 2D tissue model size, 5x5 cm\textsuperscript{2}, 63,000 nodes; sink zone radius, \( r=1 \text{ cm} \); tissue conductivity, 0.1 S/m. Excitation–contraction coupling, mitochondrial energetics, and ROS-induced ROS release model parameters were otherwise identical to those in the study by Zhou et al.\textsuperscript{17}
Experimental Protocol

Neonatal rat ventricular myocytes (NRVM) were isolated from ventricles of 2-day-old neonatal Sprague Dawley rats (Harlan Laboratories), as previously described. The procedure conformed to the protocols in the National Institutes of Health, Guide for the Care and Use of Animals (NIH publication No. 85-23, revised 1996). Cells were resuspended in Medium 199 (Invitrogen) and supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen). After 2 steps of preplating, 850,000 cells were plated on plastic of viscous coverslips (D=2.1 cm) coated with fibronectin (25 μg/mL). After 1 day, serum was reduced to 2%. Monolayers were used for experiments after 5 to 7 days in culture.

To observe changes in sarcosomellar membrane potential, monolayers were loaded with 5 μmol/L di-4-ANEPPS (4-(2-(6-(Dibutylamino)-2-naphthalenyl)ethyl)-1-(3-sulfopropyl)pyridinium hydroxide inner salt; Invitrogen) for 15 minutes, placed in the optical mapping setup, and continuously superfused with Tyrode solution consisting of (in mmol/L) 135 NaCl, 5.4 KCl, 1.8 CaCl2, 0.33 NaH2PO4, 5 HEPES, and 5 glucose. To create a metabolic sink, a custom-made local perfusion device was used to expose the center part of the monolayer to Tyrode solution containing the mitochondrial uncoupler carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP; 1 μmol/L; Sigma-Aldrich); the rest of the monolayer received only normal Tyrode solution. FCCP was intentionally used as a tool to generate a metabolic sink without inducing extensive oxidative modifications of ion channels and transporters so that experimental and model conditions were comparable. Because of the shorter AP of NRVM compared with the model, the sizes of the monolayer and central sink region in the experiments were smaller so that spatial relationships in the experiment corresponded to those in the simulations. Changes in the membrane voltage were recorded with a 464-element photodiode array (WuTech) and analyzed using software written in LabVIEW (Texas Instruments) and MATLAB (MathWorks). Giblencamide, a selective blocker of KATP channels, was used at a concentration of 10 μmol/L. Experiments were performed at 37°C.

Regional ΔΨm depolarization by local FCCP perfusion was recorded using the potentiometric fluorescent indicator TMRM (tetramethylrhodamine methyl ester; 2 μmol/L) in the dequench mode. After TMRM was loaded for 1 hour, the monolayer was imaged from above by means of a cooled CCD camera (MicroMax 1300Y; Princeton Instruments).

Results

Regional ΔΨm Depolarization Forms a Metabolic Sink

Our first objective was to determine, using modeling studies, whether regional mitochondrial depolarization can form a metabolic current sink, and if so, how the sink affects electrical wave propagation. As expected from previous single-cell simulations, increasing the fraction of ROS production in the mitochondria of myocytes in the central region resulted in abrupt spontaneous ΔΨm depolarization confined to that region (Figure 1A). The effect of regional mitochondrial depolarization on electrical wave propagation was highly dependent on the density of KATP channels (σKATP). In the absence of KATP current (IKATP), regional mitochondrial depolarization had no effect on the AP or electrical wave propagation in this simulation. With moderate KATP channel density (e.g., σKATP=0.8/μm² or 1.8/μm²), the collapse of ΔΨm caused significant shortening of APD and reduction of AP amplitude (APA) in the central region (Figure 1B). The effect on wave propagation was to dramatically shorten the wavelength in the central zone, with little effect on the wavefront (Figure 1C). When σKATP was increased to 3.8/μm², APD decreased dramatically, and APA was further reduced (Figure 1B), resulting in a thin propagating wave (Figure 1C) and a short refractory period within the sink, as demonstrated by the recovery of the j-gate of the Na channel (Figure 1D). In fact, the Na⁺ channels in the metabolic sink recovered from inactivation even before the wave outside the sink had passed through the region, making the sink vulnerable to early re-excitation. The voltage gradient at the border of the sink could, in theory, have provided a source of re-excitation of the metabolic sink; however, in this simulation, the current was not sufficient to reach the threshold for re-excitation. Increasing σKATP to values >3.8/μm² resulted in the metabolic sink becoming completely inexhaustible because of a high threshold for excitation conferred by the high background K⁺ conductance. Changing KATP channel density alone had no effect on ΔΨm.

Regional Mitochondrial Depolarization Forms a Substrate for Arrhythmias

The susceptibility to re-entry in the metabolic current sink model was explored by applying a premature S2 at or near the border of the central zone (1.8/μm²≤σKATP≤3.8/μm²). Our simulations showed that there was an S1–S2 coupling interval window (≈150–205 ms) within which S2 induced re-entrant activity. For example, as shown in Figure 2A (σKATP=3.8/μm²; Movie I in the Data Supplement), when the S1–S2 interval was 170 ms, the S2-induced excitation propagated immediately into the metabolic sink as a thin wave that broke up as it emerged from the sink region, spiraling back on itself to re-enter the sink and spawning multiple wavelets at several points near the border. This fibrillatory activity was sustained for 600 ms before dying out, terminating as a result of the no-flux boundary conditions of the model. The role of the metabolic current sink in establishing fibrillation propensity was supported by the fact that the phase singularities sustaining the turbulent electrical behavior arose initially at the border of the central zone (Figure 2B; Movie II in the Data Supplement). Similar behavior was observed for larger sizes of the metabolic sink; however, re-entry did not take place for a metabolic sink of smaller radius, for example, 0.5 cm, because by the time the S2-elicited wave propagated through the sink, the surrounding normal tissue was still refractory after the S1 propagation.

Recovery of Mitochondrial Energetics Induces Spontaneous Arrhythmias

Next, we conducted simulations to investigate the effect of recovery of ΔΨm during the repolarization phase of the mitochondrial oscillations on electrical activity in the paced tissue (1.8/μm²≤σKATP≤3.8/μm²). Recovery of ΔΨm in the metabolic sink always resulted in spontaneous wavefront generation from the back of the S1 wave, which will call wavebreak breakthrough, which propagated as a rebound wave through the metabolic sink. This rebound wave affected the electrical activity in the tissue in a sink size–dependent fashion. As shown in Figure 3 (σKATP=3.8/μm²), when the sink size was relatively small (r=0.5 cm), the wave was confined within the sink zone without breaking out (Figure 3A, top). When the sink was larger (r=1 cm), the rebound wave propagated through the sink and entered normal tissue, forming spiral waves (Figure 3A, middle; Movie III in the Data Supplement). A further increase in sink size (r=2 cm) resulted in wavebreaks.
and turbulent electrical activity (Figure 3A, bottom; Movie IV in the Data Supplement). The relationship between sink size and the induction of arrhythmias for these $\sigma_{\text{KATP}}$ values is summarized in Figure 3B.

Coupling between mitochondrial energetics and cellular electrical activity in the model is primarily through the ATP-sensitive potassium channel; hence, we examined the behavior of $I_{\text{K,ATP}}$ during recovery of the metabolic sink to better understand the mechanism of waveback breakthrough. As shown in Figure 4A for the 2 cm radius case, when the S1 wave, initiated from the left-lower corner of the model, reached the edge of the sink (at 58 ms, point a, top row), mitochondria within the sink were partially repolarized ($\Delta \Psi_m$ = 37 mV; Figure 4B), but $I_{\text{K,ATP}}$ was still activated, at 22 $\mu$A/cm$^2$ (the spatial distribution of $I_{\text{K,ATP}}$ in the sheet at the same instant of time is presented in Figure 4A, middle, whereas the temporal traces of $I_{\text{K,ATP}}$ at points a to d are depicted in Figure 4C). Although the S1 wave propagated through the sink zone, mitochondria continued to recover (Figure 4A, middle), resulting in a diminution of $I_{\text{K,ATP}}$. At 132 ms, when $\Delta \Psi_m$ had recovered to 90% of its maximal value (121 mV), $I_{\text{K,ATP}}$ was almost completely inactivated (2.4 $\mu$A/cm$^2$ at point c in Figure 4A and 4C). The rapid inactivation of $K_{\text{ATP}}$ current reversed the current dissipation by the sink, thus lowering the threshold for re-excitation. The combination of short effective refractory period and lowered threshold for firing permitted waveback breakthrough (point d), leading to a rebound wave.

To dissect the spatiotemporal determinants of the aberrant electrical behavior induced by $\Delta \Psi_m$ changes, we examined how the timing of metabolic sink recovery (relative to S1) affects the induction of spontaneous arrhythmias. Figure 5A shows the energetic state of the sink when the wavefront reaches the edge of the sink (58 ms) for various intervals between electrical stimulation and mitochondrial recovery, ES-ME, whereas Figure 5B shows the time course of $\Delta \Psi_m$ repolarization at the center of the sink. We found that when the lag was too large (eg, 154 ms) or too small (eg, 23 ms), no wavebreak or fibrillation was observed (Figure 5C). When the lag was in the range of 45 to 150 ms, irregular electrical activity was induced (Figure 5C). Particularly, 2 types of arrhythmias were observed: rebound waves, which exited the sink when the lag was between 45 and 103 ms, and fibrillation, when the lag was between 103 and 150 ms. This relationship is summarized in Figure 5D.

Spontaneous arrhythmias because of metabolic recovery did not always originate directly from the waveback. In simulations where the conductivity in the central zone was decreased to reflect the possible gap junction sensitivity to the level of ATP, the stimulus-induced wavefront could not penetrate the sink zone because additional excitatory current was required, as shown in Figure 6 ($\sigma_{\text{KATP}}$=3.8/µm$^2$; $r$=2 cm; sink zone...
conductivity, 0.03 S/m). Instead, rebound waves were initiated at the edge of the sink at sites of high transmembrane voltage gradient when the excitation threshold decreased in the sink during mitochondrial recovery. In this example, conduction velocity was lower in the sink compared with that in Figure 3; therefore, the rebound wave took longer to propagate through it, allowing for the surrounding normal tissue to recover from the S1 wave, resulting in re-entry even though the radius was smaller than that shown to be resistant in Figure 3B. Thus, a decrease in gap junctional conductance, which was incorporated into the model to reproduce the magnitude of wavefront slowing observed in the experiments (Figure 7B), also has the effect of decreasing the minimum size of the metabolic sink supporting re-entry.

Figure 4. Electrical wave, mitochondrial inner membrane potential ($\Delta \Psi_m$) recovery, and $I_{\text{K,ATP}}$ during waveback breakthrough. A, Propagation of the electrical wave (top), recovery of $\Delta \Psi_m$ (middle), and $I_{\text{K,ATP}}$ current (bottom). B, The time course of $\Delta \Psi_m$ recovery at the sink center. C, Inactivation of $I_{\text{K,ATP}}$ currents accompanying mitochondrial repolarization. $\sigma_{\text{K,ATP}}=3.8/\mu m^2, r=2$ cm.

Figure 5. Effect of lag time between the electrical stimulus and the recovery of mitochondrial energetics (ES-MElag) on re-entry induced by the metabolic sink. A, Mitochondrial inner membrane potential ($\Delta \Psi_m$) distribution at the time when S1 reaches the edge of the metabolic sink as ES-MElag is varied. B, Dynamics of $\Delta \Psi_m$ recovery at the metabolic sink center with different lag times. C, Recovery of $\Delta \Psi_m$ induces rebound propagation or fibrillation depending on ES-MElag. D, Summary of the effect of lag times on the induction of arrhythmias. $\sigma_{\text{K,ATP}}=3.8/\mu m^2, r=2$ cm.
Regional $\Delta \Psi_m$ Depolarization Induces Abnormal Electrical Activity in NRVM Monolayers

Local perfusion\(^25\) of the center part (D=0.5 cm) of the monolayer with FCCP induced regional $\Delta \Psi_m$ depolarization (Figure 7A) and resulted in a fast decrease in both APA and APD\(_{50}\) (Figure 7C and 7D; Figure II in the Data Supplement). As a consequence of $\Delta \Psi_m$ depolarization, wavelength shortened, and wave slowing was also observed (Figure 7B, top). Simulations including both $K_{ATP}$-dependent coupling and decreased gap junctional conductance closely matched these results (Figure 7B, bottom). The heterogeneity of refractoriness and slower conduction observed in the NRVM monolayers after induction of the metabolic sink occasionally led to re-entry. Movie V in the Data Supplement and Figure 7E show such an event in a monolayer paced at 4 Hz. Spiral waves continued for several minutes after pacing was turned off. Also, heterogeneity in refractoriness and conduction, because of reperfusion of the metabolic sink with normal physiological solution to restore $\Delta \Psi_m$, caused transient or sustained re-entry. Figure 7F shows formation of a transient re-entrant wave in a monolayer as the sink started to regain excitability on FCCP washout. Overall, re-entry occurred in 6 of 10 monolayers paced at 1 to 4 Hz (4 monolayers during FCCP perfusion and 4 during washout).

$K_{ATP}$ channels played a key role in the electrophysiological changes induced by the metabolic sink. Glibenclamide (10 $\mu$mol/L) blunted the APA decrease and largely prevented the APD shortening (Figure 7C and 7D). Consequently, in contrast to FCCP perfusion alone, glibenclamide preserved excitability of the sink area. Transient re-entry occurred for 1 to 2 minutes in only 2 of 6 monolayers (1 monolayer during FCCP perfusion and 1 during washout). The effect of glibenclamide was even more pronounced at room temperature (Figure III in the Data Supplement).

To study the effect of sink size on the occurrence of re-entry, in another set of experiments (21 monolayers), a larger area (D=1.2 cm) of the NRVM monolayer was perfused with FCCP, and thus, a larger metabolic sink was induced. In this case, re-entry occurred during FCCP perfusion in response to an S2 stimulus ($S1-S2=200$ ms) on the border of the large sink in 3 of 3 monolayers tested, whereas extrastimuli at the border of the smaller sink did not cause re-entry in any monolayer.
In the absence of an S2 stimulus, spontaneous activity was observed to be initiated close to the edge of the sink and led to re-entry in 2 of 18 monolayers even at the low pacing rate of 1 Hz (Movie VI in the Data Supplement).

Discussion

In this study, 2-dimensional simulations and experiments in cardiac cell monolayers demonstrated that the formation of a metabolic sink, induced by regional mitochondrial depolarization, profoundly affects electrical activity in the tissue. The overall influence of energetic collapse is to decrease APA and APD, decreasing wavelength and introducing regions of short refractory period that facilitate re-entry. In both computational and experimental models, premature beats at locations surrounding the metabolic sink resulted in spiral wave re-entry and fibrillation in a sink size–dependent manner. Furthermore, our simulations showed that metabolic recovery could result in spontaneous electrical instability through a novel mechanism involving waveback breakthrough, depending on the size of the metabolic sink and the timing of ΔΨ\textsubscript{m} recovery with respect to wavefront arrival.

Sarcolemmal K\textsubscript{ATP} channels are activated by a decrease in cytosolic ATP and mediate a weakly inwardly rectifying background K\textsuperscript{+} current. Mitochondrial ΔΨ\textsubscript{m} depolarization can potentiate K\textsubscript{ATP} channel opening because uncoupling of oxidative phosphorylation results in a reversal of the mitochondrial ATP synthase, accelerating the depletion of intracellular ATP. Increased background K\textsuperscript{+} conductance through K\textsubscript{ATP} channels pulls the resting membrane potential (E\textsubscript{m}) close to the equilibrium potential for K\textsuperscript{+} (E\textsubscript{K}). After 10 minutes of ischemia, resting membrane potential is, in fact, equal to E\textsubscript{K}\textsuperscript{-30} K\textsubscript{ATP} current activation accounts for most of the AP shortening during the early phase of ischemia, as evidenced by the ability of K\textsubscript{ATP} channel inhibitors such as glibenclamide to prevent shortening of the AP duration during the first 10 minutes of ischemia. Concomitant with AP shortening, there is a monotonic decrease in APA and upstroke velocity during the first 10 minutes of ischemia, which can be partially prevented by glibenclamide treatment, suggesting that K\textsubscript{ATP} channels may contribute to both the AP shortening and early conduction slowing in the ischemic heart. Our experiments supported this idea by showing that blocking sarcolemmal K\textsubscript{ATP} channels blunted the loss of APA and APD during mitochondrial depolarization and lowered the chance of re-entry in monolayers of cardiomyocytes. The effect of glibenclamide was even more evident at room temperature (Figure III in the Data Supplement) likely because of temperature-dependent alterations in metabolic flux. In the intact heart, with longer durations of mitochondrial dysfunction, however, other mechanisms besides K\textsubscript{ATP} activation come into play, including a further decrease in conduction velocity, gap junctional uncoupling, catecholamine release (at 15–20 minutes), increases in Ca\textsuperscript{2+} (2- to 3-fold) and Mg\textsuperscript{2+} (>3-fold), and a second phase of ATP decline.

The present simulations focused primarily on how K\textsubscript{ATP} channel activation by mitochondrial depolarization alters the excitabile substrate and can lead to arrhythmias. The model was designed to represent the effects of mitochondrial dysfunction during periods of high oxidative stress, such as reperfusion. Previously, we showed that in guinea pig hearts, reperfusion after 30 minutes of global ischemia evokes re-entrant arrhythmias, and that treatment with a compound that prevents or reverses RIRR-mediated mitochondrial depolarization (4′-chlorodiazepam) eliminates postischemic ventricular fibrillation and improves AP recovery. Similarly, we have shown that significant spatiotemporal heterogeneity of ΔΨ\textsubscript{m} is present during ischemia–reperfusion in isolated hearts. Furthermore, we have demonstrated that in normoxic hearts exposed to oxidative stress (reduced glutathione depletion with diamide), an increase in the incidence of ventricular arrhythmias occurs in parallel with heterogeneous ΔΨ\textsubscript{m} loss in clusters of myocytes. The arrhythmias and the mitochondrial collapse of ΔΨ\textsubscript{m} were both inhibited by 4′-chlorodiazepam.

Because mitochondrial RIRR activates K\textsubscript{ATP} current and shortens APD, a major assumption of the metabolic sink hypothesis was that heterogeneous repolarization was responsible for the enhanced susceptibility to re-entry in these studies. The computational and experimental tests described in the present work demonstrate that regional mitochondrial depolarization can indeed increase the susceptibility to re-entry in response to an extrastimulus. Furthermore, simulations uncovered a novel ectopic trigger mechanism of fibrillation, occurring spontaneously during ΔΨ\textsubscript{m} recovery. The latter mechanism is a model prediction that remains to be demonstrated in experimental systems or intact hearts. This will require high-speed, high-resolution imaging of both mitochondrial and sarcolemmal membrane voltages simultaneously. Nevertheless, in addition to more well-established mechanisms of spontaneous automaticity (ie, early- and delayed-afterdepolarizations), the mechanisms of wavebreak breakthrough and rebound waves could contribute to the high rate of re-entrant arrhythmias observed when mitochondria are recovering and the spatiotemporal heterogeneity of ΔΨ\textsubscript{m} is high during early reperfusion.

The effect of K\textsubscript{ATP} channel inhibition on ischemia–reperfusion injury and arrhythmogenesis is variable and species dependent. For example, in mice treated with the K\textsubscript{ATP} channel blocker HMR1098, ischemia causes a much more rapid contracture of the isolated perfused heart (within 5 minutes of the onset of ischemia) compared with untreated hearts. In this species, APs are already short and the heart rate is extremely high (=600 bpm), which could potentially explain why K\textsubscript{ATP} channel activation during ischemia is essential to prevent a tetanus-like depolarization of the membrane potential under metabolic stress. In contrast, in larger animals with slower heart rates and longer AP plateau potentials (eg, guinea pig, dogs, humans), glibenclamide treatment does not accelerate ischemic contracture, although it does prevent the protective effects of ischemic or chemical preconditioning. In fact, K\textsubscript{ATP} channel inhibition has been shown to prevent ventricular fibrillation after acute myocardial infarction in noninsulin-dependent diabetic patients. Furthermore, K\textsubscript{ATP} channel inhibition effectively prevented arrhythmias induced by the combination of acute myocardial ischemia and exercise in dogs with healed myocardial infarctions. Notably, the antiarrhythmic effect was observed with compounds (HMR1883 and congeners) that were designed to inhibit the heart sarcolemmal K\textsubscript{ATP} channel selectively without affecting pancreatic insulin release or mitochondrial K\textsubscript{ATP} channels.
Thus, consistent with the present findings, available evidence supports the arrhythmogenic role of $K_{\text{ATP}}$ in association with ischemia–reperfusion injury in large animals. Furthermore, enhanced arrhythmogenicity related to $K_{\text{ATP}}$ activation was confirmed in a recent study in explanted failing and nonfailing human hearts.36

Previous works, from Noujaim et al37 and Jalife,38 have emphasized how increased inward rectifier, delayed rectifier,39 and more recently, hERG40 $K^+$ current expression can increase the susceptibility to re-entry in structurally normal, well-perfused hearts. This arrhythmogenic effect is primarily caused by AP shortening and increased rotor frequency. Conditions of ischemia–reperfusion are an even more extreme example of an acute and dramatic increase in a weak inward rectifier background current ($U_{K_{\text{ATP}}}$). Thus, the general principles of rotor stabilization and increased fibrillation risk apply, although many other factors also come into play, including changes in transmembrane K+, Na+, and Ca2+ gradients, pH, ATP, Mg2+, and tissue conductivity. In fact, as the experiments with the mitochondrial uncoupler show, mitochondrial Δψm collapse affects the AP and the propagating wavefront in a manner that is more complex than expected simply from $K_{\text{ATP}}$ channel activation, although this mitochondrial intervention induces less oxidative stress than ischemia–reperfusion per se (data not shown). More pronounced effects on conduction velocity and APA in the experiments than in the simulations are likely to be because of metabolic regulation of targets not yet represented in the computational model. Importantly, although the FCCP-induced metabolic sink evokes less oxidative stress than other metabolic or oxidative challenges and in that sense behaves more like the present computational model, this does not mean that multiple targets of oxidative stress would not play a role in arrhythmogenesis when RIRR underlies the formation of metabolic sinks in the heart. We continue to define these additional targets in ongoing studies.

Additional model development will be required to more completely describe the changes in the electrophysiological substrate induced by ischemia–reperfusion. Nevertheless, by incorporating mitochondrial energetics into the tissue electrical model, the first step has been taken to begin to correlate metabolic dysfunction with downstream effects on whole heart electrophysiology. This will be vital for understanding how metabolic remodeling, which is present in metabolic syndrome, hypertrophy, heart failure, and diabetes mellitus, influences the electrophysiological substrate and potentially contributes to sudden cardiac death. Furthermore, the findings underscore the importance of considering mitochondria as targets of therapeutic intervention not only for preserving healthy cardiac muscle during stress but also for the arrhythmias associated with cardiovascular disease.

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Mitochondrial Depolarization and Re-Entry

CLINICAL PERSPECTIVE

Ischemia–reperfusion injury is known to trigger potentially fatal arrhythmias involving re-entrant electrical activity, but the mechanisms leading from energetic impairment to arrhythmias are incompletely understood. Here, using a combined computational and experimental approach, we examine how regional depolarization of mitochondrial inner membrane potential can promote re-entry in a cardiac cell monolayer through the formation of a metabolic current sink. A 2-dimensional cardiac tissue computational model revealed that oxidative stress–mediated collapse of mitochondrial inner membrane potential and the consequent activation of ATP-sensitive K+ channels in part of the monolayer increases the vulnerability to re-entry by increasing the dispersion of repolarization of the electrical substrate. Similar effects were observed in a cardiac cell monolayer exposed to a chemical uncoupler of mitochondrial oxidative phosphorylation. The findings implicate mitochondria as potential targets for antiarrhythmic therapy.
Effects of Regional Mitochondrial Depolarization on Electrical Propagation: Implications for Arrhythmogenesis
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Supplemental Fig 1. General scheme of the E-C coupling, Mitochondrial Energetics and ROS Induced ROS Release (ECME-RIRR) model. The electrophysiological module describes the major ion channels underlying the action potential and the processes involved in Ca^{2+} handling, and the inner mitochondrial membrane channels. The mitochondrial module accounts for the major components of mitochondrial energetics. The RIRR module describes ROS production, transport and scavenging. The mitochondrial energetics and ROS are linked to cellular electrical activity through the energy sensitive K_{ATP} current. This model displays many of the features observed in whole-cell experiments carried out in guinea pig myocytes. In particular, the ECME-RIRR model recapitulates the close coupling between mitochondrial energy state, ROS bursting, and APD shortening observed in myocytes subjected to oxidative stress. It is the first cardiac cell model to consider the links between ion fluxes, energy-consuming reactions (e.g. myosin ATPase), energy production, and ROS balance, thereby allowing us to investigate how mitochondrial dysfunction translates into electrophysiological alterations that could lead to...
arrhythmias. It is worthy to point out that various experimental studies have demonstrated that many ion channels and transporters are sensitive to both redox state and phosphorylation state. However, as our major objective was to investigate whether and how mitochondrial dysfunction, particularly depolarization and increased ATP turnover associated with KATP channel activation, could influence AP propagation in cell monolayers, only some of these components are linked to ATP/ADP levels in the present model (i.e., the KATP channel and the energy-dependent pumps and ATPases).

Supplemental Fig 2. Changes in action potential in the sink area due to FCCP perfusion and washout.

Supplemental Fig 3. Effect of local perfusion with FCCP (1µM) on optically measured APA and APD50 in the sink area with/without glibenclamide (10µM) at room temperature.
SUPPLEMENTAL MATERIAL

Model simulation protocol

The tissue model was paced (S1) at 1 Hz from the lower left corner of the sheet to reach steady state. In the first set of simulations, regional mitochondrial depolarization was induced in all cells within a central circular region of radius 1 cm (Fig 1A). This was achieved by increasing fractional ROS production by the electron transport chain in these cells, expressed as a percentage of the total oxygen consumption rate (a.k.a., the *shunt*), from the physiological value of 2%, to 14%. This forced the mitochondria in the central zone to undergo a dynamic bifurcation towards sustained oscillations, while mitochondria in the normal zone (shunt = 2%) remained polarized during the entire simulation. Simulations with other radii of the metabolic sink, 0.5 and 2 cm were also conducted. To determine the susceptibility of the metabolic-sink substrate to reentry, a single-pulse premature stimulus, S2, was applied near the border of the metabolic sink at various coupling intervals (with respect to S1).

The second set of simulations investigated the effect of recovery of ΔΨ_m in the metabolic sink on electrical wave propagation during the repolarization phase of the mitochondrial oscillations, in the absence of extrastimuli. The lag time between the electrical stimulus and the recovery of mitochondrial energetics (ES-ME_lag) was defined as the time interval between the initialization of S1 and 90% recovery of ΔΨ_m at the sink center. In a subset of the simulations, the tissue conductivity in the sink zone was decreased from the normal value of 0.1 S/m to values as low as 0.03S/m, to reflect the fact that gap junctions are also sensitive to the level of ATP^2.

References

Movie Legends

**Movie 1.** Sustained fibrillation in the tissue model (1 Hz pacing) evoked by a second stimulus (S2) near the border of the metabolic sink. The S1 S2 time interval is 170 ms. $\sigma_{\text{KATP}} = 3.8/\mu\text{m}^2$, $r = 1$ cm.

**Movie 2.** The same activity as in Movie 1, but presented as a distribution of action potential phase. Phase singularities are shown as magenta dots.

**Movie 3.** $\Delta\Psi_m$ repolarization in the metabolic sink induced a rebound excitation resulting in reentry. $\sigma_{\text{KATP}} = 3.8/\mu\text{m}^2$, $r = 1$ cm.

**Movie 4.** $\Delta\Psi_m$ repolarization in the metabolic sink induced a rebound excitation resulting in fibrillation. $\sigma_{\text{KATP}} = 3.8/\mu\text{m}^2$, $r = 2$ cm.

**Movie 5.** Formation of reentry due to induction of metabolic sink by local FCCP (1µM) perfusion in an NRVM monolayer. Stimulation at 4Hz. Movie is slowed 4X.

**Movie 6.** Spontaneous activity on the border of the sink provides a unidirectional block resulting in reentry. Bipolar point stimulation: 1 Hz