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Review

From mitochondrial dynamics to arrhythmias

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ABSTRACT

The reactive oxygen species (ROS)-dependent mitochondrial oscillator described in cardiac cells exhibits at least two modes of function under physiological conditions or in response to metabolic and oxidative stress. Both modes depend upon network behavior of mitochondria. Under physiological conditions cardiac mitochondria behave as a network of coupled oscillators with a broad range of frequencies. ROS weakly couples mitochondria under normal conditions but becomes a strong coupling messenger when, under oxidative stress, the mitochondrial network attains criticality. Mitochondrial criticality is achieved when a threshold of ROS is overcome and a certain density of mitochondria forms a cluster that spans the whole cell. Under these conditions, the slightest perturbation triggers a cell-wide collapse of the mitochondrial membrane potential, $\Delta\psi_m$, visualized as a depolarization wave throughout the cell which is followed by whole cell synchronized oscillations in $\Delta\psi_m$, NADH, ROS, and GSH. This dynamic behavior scales from the mitochondrion to the cell by driving cellular excitability and the whole heart into catastrophic arrhythmias. A network collapse of $\Delta\psi_m$ under criticality leads to: (i) energetic failure, (ii) temporal and regional alterations in action potential (AP), (iii) development of zones of impaired conduction in the myocardium, and, ultimately, (iv) a fatal ventricular arrhythmia.

Contents

1. Introduction

Under metabolically stressful conditions such as substrate deprivation or oxidative stress, the role of mitochondrial function becomes a key arbiter of life and death at the cellular and organ level. While under normal physiological conditions the availability of energy is fine tuned to match changes in energy demand, under stress this is not the case. Most myocardial ATP production occurs in the mitochondria through oxidative phosphorylation, and most

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ATP utilization occurs at the myofibrils (Cortassa et al., 2006; Saks et al., 2007; Wallimann et al., 2007). Direct measures of ATP synthesis through creatine kinase in the human heart demonstrated a deficit in energy supply in clinical heart failure (Weiss et al., 2005). This reduction in ATP synthesis through CK is cardiac-specific and occurs in mild-to-moderate heart failure before a significant reduction in ATP can be detected.

The remarkable non-linear properties of the mitochondrial network, and of the heart itself, make them prone to the appearance of critical phenomena and bifurcations leading to self-organized, emergent, behavior. A dramatic example of the latter is the succession of failures shown to escalate from the mitochondrial network to the whole heart resulting in reperfusion-related arrhythmias after ischemic injury, and eventually the death of the organism (Akar et al., 2005; Aon et al., 2006a; O'Rourke et al., 2005). Mitochondria from heart cells act as a network of coupled oscillators, capable of producing frequency- and/or amplitude-encoded reactive oxygen species (ROS) signals under physiological conditions (Aon et al., 2006b, 2007b, 2008). This intrinsic property of the mitochondria can lead to a mitochondrial 'critical' state, i.e., an emergent macroscopic response manifested as a generalized $\Delta\psi_m$ collapse followed by synchronized oscillation in the mitochondrial network under stress (Aon et al., 2004). The large amplitude $\Delta\psi_m$ depolarization and bursts of ROS have widespread effects on all subsystems of the cell including energy-sensitive ion channels in the plasma membrane, producing an effect that scales to cause organ level electrical and contractile dysfunction. Mitochondrial ion channels appear to play a key role in the mechanism of this non-linear network phenomenon and hence are a potential target for therapeutic intervention.

The loss of $\Delta\psi_m$ is among the leading factors causing a rapid impairment of mitochondrial and cellular function that may result into necrotic or apoptotic cell death (Aon et al., 2007a; Gustafsson and Gottlieb, 2008; Slodzinski et al., 2008). Thus, maintaining $\Delta\psi_m$ is of paramount importance. Oxidative stress is a major pathophysiological route to the collapse of $\Delta\psi_m$ (Aon et al., 2003, 2004; Brady et al., 2004; Zorov et al., 2000). The toxic effects of ROS are kept in check throughout our lives by balancing the natural rates of ROS production with sophisticated antioxidant defense systems. If this balance between ROS production and ROS scavenging is disrupted, serious and often irreversible cell damage occurs (Halliwell, 1997). One such important pathological situation in the heart is reperfusion following ischemia, when ROS production accelerates and the detoxification systems are overwhelmed, resulting in the consumption of antioxidants and an increase in free radical concentrations (Aon et al., 2007a; Lucas and Szweda, 1998; Marczin et al., 2003; Slodzinski et al., 2008). This is the period during which $\Delta\psi_m$ is most likely to become unstable, representing a major decision point between cell life or death.

2. Mitochondrial physiology and ion channels

The oxidation of fuels (e.g., fatty acids and glucose) leads to acetyl-CoA, the common substrate for the Krebs cycle which, in turn, drives the production of the reducing equivalents NADH and $FADH_2$. Electrons are passed to the electron transport chain, where coupled redox reactions mediate proton translocation across the inner membrane to establish a proton-motive force (PMF) composed of an electrical potential and pH gradient that drives ATP synthesis by the mitochondrial ATP synthase. The PMF is the major driving force for proton influx and is used by the mitochondrial ATP synthase (F1F0 ATPase) to produce ATP, which is exported to the cytosol via the adenine nucleotide translocase.

Maximum coupling between proton pumping by the respiratory chain and the phosphorylation of ADP is obtained when the leak of protons across the membrane is minimized. The energy dissipated by the increased ion permeability stimulates NADH oxidation, proton pumping, and respiration. This increased permeability can be carried out by ion-selective or non-selective mitochondrial channels that dissipate energy and alter the ionic balance and volume of the mitochondrial matrix. These ionic movements may be partly compensated by antiporters coupled to H^+ movement. Concomitant stimulation of NADH production is required to compensate for the higher rates of respiration, or else a mismatch in energy supply and demand will occur.

Several inner membrane ion channels have been described, and their pro-life or -death effects highlighted (reviewed in Aon et al. (2006a, 2007b), Brady et al. (2006) and O'Rourke et al. (2007)). Among them are the inner membrane anion channel (IMAC), a reversible channel activated under moderate oxidative stress, and the permeability transition pore (PTP), a large, non-selective, ion channel responsible for irreversible $\Delta\psi_m$ depolarization under high oxidative stress conditions. These ion channels have been described on the mitochondrial inner membrane, and have been shown to be responsible for fast mitochondrial depolarization. However, the reversibility of $\Delta\psi_m$ depolarization depends on which of these two channels is activated. As a matter of fact, we have recently shown that IMAC and PTP open sequentially as a function of oxidative stress and matrix and cytoplasmic redox potentials. Under moderately low ratios of reduced glutathione (GSH) to oxidized glutathione (GSSG), a moderate increase in ROS activates IMAC and oscillations in mitochondrial inner membrane potential ($\Delta\psi_m$) can be sustained. These can be reversed by inhibition of this channel. However, at more oxidized redox potentials, permeability transition pore (PTP) opening leads to irreversible $\Delta\psi_m$ collapse (Aon et al., 2007a).

An IMAC was originally described in isolated mitochondria and was shown to be inhibited by cationic amphiphiles including peripheral (mitochondrial) benzodiazepine receptor (mBzR) ligands (Beavis, 1989; Beavis and Garlid, 1987). Subsequently, single channel patch-clamp studies of mitoplasts have provided evidence that anion channels are present on the inner membrane, the most common being the outwardly rectifying 108 pS (or "centum-picosiemen") anion channel which is inhibited by mBzR antagonists. We showed that PK11195, an isoquinoline carboxamide mBzR ligand, or a structurally different mBzR ligand, 4′-chlorodiazepam (4′Cl-DZP, or its synonym Ro5-4864) could acutely inhibit mitochondrial oscillations (Aon et al., 2003; O'Rourke, 2000). These inhibitors prevented ROS accumulation in the mitochondrial network, but actually potentiated ROS accumulation in the small laser-flashed region of the cell, leading to the proposal that IMAC might also be an efflux pathway for superoxide anion, $O_2^{\bullet -}$, from the matrix, since the latter is membrane impermeable. Moreover, induction of mitochondrial $\Delta\psi_m$ depolarization by FGIN-1-27, an agonist that binds selectively to the mBzR, reinforced the idea that this receptor, which is thought to be present on the mitochondrial outer membrane, may be modulating IMAC (Akar et al., 2005; Aon et al., 2003). The data concerning the mechanism of the mitochondrial oscillator in heart cells, are consistent with a role for IMAC, rather than PTP, in both $\Delta\psi_m$ depolarization and $O_2^{\bullet -}$ efflux (see Aon et al. (2008) for a review).

3. Mitochondrial networks in physiology and pathophysiology

3.1. The ROS-dependent mitochondrial oscillator

When mitochondria oscillate in living cells, the asymmetry of the $\Delta\psi_m$ depolarization–repolarization cycle is consistent with the behavior exhibited by relaxation oscillators that possess slow and