Mitochondrial Oscillations in Physiology and Pathophysiology

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Abstract

Oscillations in chemical reactions and metabolic pathways have historically served as prototypes for understanding the dynamics of complex nonlinear systems. This chapter reviews the oscillatory behavior of mitochondria, with a focus on the mitochondrial oscillator dependent on reactive oxygen species (ROS), as first described in heart cells. Experimental and theoretical evidence now indicates that mitochondrial energetic variables oscillate autonomously as part of a network of coupled oscillators under both physiological and pathological conditions. The physiological domain is characterized by small-amplitude oscillations in mitochondrial membrane potential ($\Delta \Psi_m$) showing correlated behavior over a wide range of frequencies, as determined using Power Spectral Analysis and Relative Dispersion Analysis of long term recordings of $\Delta \Psi_m$. Under metabolic stress, when the balance between ROS generation and ROS scavenging is perturbed, the mitochondrial network throughout the cell locks to one main low-frequency, high-amplitude oscillatory mode. This behavior has major pathological implications because the energy dissipation and cellular redox changes that occur during $\Delta \Psi_m$ depolarization result in suppression of electrical excitability and $\text{Ca}^{2+}$ handling, the two main functions of the cardiac cell. In an ischemia/reperfusion scenario these alterations scale up to the level of the whole organ, giving rise to fatal arrhythmias.

Introduction

The study of oscillatory phenomena in physics, chemistry and biology has fascinated scientists for centuries. For example, the synchronization of pendulum clocks as coupled oscillators was described by Huygens in 1665, and in biological systems, Kaempfer wrote of the synchronized blinking of fireflies that he had observed on a visit to Siam in 1680 (see refs. 1, 2 for historical reviews). In the 20th century, Van der Pol modeled the electrophysiological properties of the heart as collection of coupled relaxation oscillators and other examples of electrical, biochemical, and ionic oscillators in physiology abound.

The Russian scientist B.P. Belousov demonstrated that complex temporal and spatial dynamics can be observed in the simple chemical reaction of citric acid/bromate/cerium. He had difficulty publishing the work when first submitted in 1951, but after Zhabotinsky repeated Belousov’s experiments, the Belousov-Zhabotinsky reaction grew to became a prototype for the theoretical and experimental study of oscillators. In the same era, oscillations in enzyme-catalyzed biochemical pathways were reported. Duysens and Amesz published the

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first observation of biochemical oscillations of reduced pyridine nucleotides in intact cells and these findings were followed by the elucidation of a glycolytic oscillator in yeast11,12 and in cell-free extracts from various sources.13-18 This triggered an explosion of interest in the elucidation of the mechanism(s) of biological oscillators (see refs. 4 and 19 for early reviews, see Lloyd, D, this volume). In yeast, this interest has continued throughout the years with new exciting findings.7,20-22

Oscillations in ion fluxes, respiration, and mitochondrial volume were noted 40 years ago in several laboratories23-25 and Chance and Yoshioka26 demonstrated that they could be sustained over many cycles. Others have also observed mitochondrial oscillations triggered by divalent cations.27-30 In these earlier studies, the oscillations were typically induced by an increase in cation cycling (e.g., by adding valinomycin) in isolated energized mitochondria and usually took the form of damped sinusoidal oscillations, the damping corresponding to a loss of synchrony among mitochondria in the population over time.31-33

In 1994, our laboratory reported that substrate deprivation could induce spontaneous low frequency oscillations in sarcolemmal K+ currents, action potential duration and excitation-contraction coupling in adult cardiomyocytes.34 These oscillations were associated with cycles of oxidation and reduction of the intracellular NADH pool. At the time, we proposed that the mechanism could involve the well-known glycolytic oscillator, which could entrain oxidative phosphorylation, but we could not exclude mitochondria as the source of oscillation. Subsequent investigations revealed that the oscillations were associated with mitochondrial flavoprotein redox transients and waves of mitochondrial membrane potential (ΔΨm) depolarization,35 which shifted our view to a mitochondrial mechanism. More recently, we have investigated in detail the mechanisms involved in triggering and maintaining synchronized, self-sustaining oscillations of bioenergetics in the mitochondrial network of intact cardiac cells, aided by the discovery that they could be reproducibly triggered by laser-induced depolarization of just a few mitochondria in the cell.36

Here, we review the properties of mitochondrial oscillators described previously and present a comprehensive account of our theoretical and experimental studies of the reactive oxygen species (ROS)-dependent mitochondrial oscillator induced by metabolic stress in heart cells. Moreover, we put forward the idea, based on relative dispersion and power spectral analysis that coupled oscillation is an inherent property of the mitochondrial network under physiological conditions as well. Understanding the basic mechanism of mitochondrial oscillation is shown to provide fundamental new insights into the origins of post-ischemic electrical and contractile dysfunction in the whole heart and also suggests a novel frequency- and amplitude-encoded ROS signalling function for mitochondria.

**Early Descriptions of Mitochondrial Ionic Oscillations**

Oscillations in ion flux across the inner membrane of isolated liver or heart mitochondria have been known since the 1960s (reviewed in ref. 32). Chance and Yoshioka reported persistent sinusoidal oscillations of H+ and K+ induced by valinomycin in the presence of oxygen, monitored with ion-selective glass electrodes.26 Initially, K+ is taken up by the mitochondria (seen as a decrease of K+ in the medium) and this is then accompanied by H+ extrusion. The K+ uptake results in mitochondrial swelling, as judged photometrically and by electron microscopy.31 Oscillations come to a halt after oxygen in the medium depletes and the ionic changes reverse, i.e., H+ concentration outside the mitochondria decreases and the K+ concentration increases.26,31 The damping of the ionic oscillations in isolated mitochondria is a function of the external pH, the K+/H+ ratio, and ADP, and the F1F0 ATPase is required, since oligomycin blunts the oscillations.26,31

Subsequent work on mitochondrial suspensions showed that the addition of a pulse of Sr2+ (a Ca2+ analogue that is efficiently transported across mitochondrial membranes) could trigger sustained oscillations in fluxes of divalent ions (reviewed in ref. 27). The shape, amplitude and frequency of oscillation were sensitive to the type of substrate. The oscillatory period ranged