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Regulation of mitochondrial Ca^{2+} and its effects on energetics and redox balance in normal and failing heart

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Abstract Ca^{2+} has been well accepted as a signal that coordinates changes in cytosolic workload with mitochondrial energy metabolism in cardiomyocytes. During increased work, Ca^{2+} is accumulated in mitochondria and stimulates ATP production to match energy supply and demand. The kinetics of mitochondrial Ca^{2+} ($[Ca^{2+}]_m$) uptake remains unclear, and we review the debate on this subject in this article. $[Ca^{2+}]_m$ has multiple targets in oxidative phosphorylation including the F1/FO ATPase, the adenine nucleotide translocase, and Ca^{2+}-sensitive dehydrogenases (CaDH) of the tricarboxylic acid (TCA) cycle. The well established effect of $[Ca^{2+}]_m$ is to activate CaDHs of the TCA cycle to increase NADH production. Maintaining NADH level is not only critical to keep a high oxidative phosphorylation rate during increased cardiac work, but is also necessary for the reducing system of the cell to maintain its reactive oxygen species (ROS) — scavenging capacity. Further, we review recent data demonstrating the deleterious effects of elevated Na^+ in cardiac pathology by blunting $[Ca^{2+}]_m$ accumulation.

Keywords Mitochondrial Ca^{2+} handling · Cardiac energy metabolism · Redox balance · Oxidative phosphorylation · Heart failure

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Introduction

The workload of the heart varies constantly and requires continuous and rapid matching of ATP supply to maintain its normal function. As a result, fine control of mitochondrial respiration is critical to meet the energy demands of cardiac muscle. The regulation of ATP synthesis has been intensively studied for decades, yet the mechanism of mitochondrial respiratory control in the heart is still not well understood. The classical model of feedback control by ADP and Pi is indisputable in isolated mitochondria (Chance and Williams 1955), but its role in cardiac mitochondrial energetics has been difficult to demonstrate in intact hearts because the total levels of the high energy phosphates appear constant for a wide range of workloads (Neely et al. 1972; Balaban et al. 1986; Katz et al. 1989; Robitaille et al. 1990; Weiss et al. 1990; Schaefer et al. 1992). This is likely to be due to inadequacies of measuring the local ADP and Pi levels at the site of acceptor control in the matrix, the F_1F_0 ATPase, since it is clear that mitochondrial ADP and Pi entry must increase dramatically in direct proportion to myosin ATPase action during contractile work. Nevertheless, several alternative models involving parallel activation of NADH production and electron transport by Ca^{2+} have been proposed (Denton and McCormack 1990; Korzeniewski 1998; Balaban 2002). In such models, Ca^{2+} acts as the primary signal that coordinates changes in cytosolic workload with mitochondrial energy metabolism in cardiomyocytes. To be such a signal, Ca^{2+} needs to meet three criteria: first, the change in cytosolic Ca^{2+} ($[Ca^{2+}]_c$) must correlate with changes in workload and ATP consumption; second, Ca^{2+} must be able to regulate ATP production in mitochondria; and third, changes in $[Ca^{2+}]_c$ cycling must be linked to changes in mitochondrial Ca^{2+} ($[Ca^{2+}]_m$). A "Ca^{2+} only" parallel model fails on the first

criterion because large changes in work can occur without significant changes in cytosolic Ca^{2+} via the Frank-Starling mechanism (Saks et al. 2006). Hence, physiological energy supply and demand matching must involve a balance of demand-led and upstream regulatory mechanisms.

Ca^{2+} plays a central role in the physiology of cardiac muscle. Ca^{2+} entry via the L-type Ca^{2+} channel triggers the opening of RyRs on the SR and induces a release of Ca^{2+} from the internal store. The concomitant rise of $[Ca^{2+}]_c$ activates cardiac contraction by binding to troponin C. $[Ca^{2+}]_c$ is then removed through the SR Ca^{2+} pump or extruded from the cell via the sarcolemmal Na^+/Ca^{2+} exchanger (NCX). Generally, increased cardiac work (except for the Frank-Starling mechanism) is associated with a higher amplitude and/or frequency of the $[Ca^{2+}]_c$ transient, and therefore increased Ca^{2+} cycling is correlated with more ATP consumption. In cardiac myocytes, cytosolic ATP is hydrolyzed by three major consumers: myosin ATPase, SR Ca^{2+}-ATPase, and Na^+/K^+ ATPase, among which the first two are activated by Ca^{2+}. Na^+/K^+ ATPase indirectly modulates Ca^{2+} cycling because of its role in determining the driving force for Na^+ and Ca^{2+} transport through NCX. In the mitochondrial matrix, Ca^{2+} has been suggested to play an important role in energetics by activating the F1/FO ATPase (Territo et al. 2000), the adenine nucleotide translocase (ANT) (Moreno-Sanchez 1985) and several Ca^{2+} sensitive dehydrogenases (CaDH) in the tricarboxylic acid (TCA) cycle, including pyruvate dehydrogenase, 2-oxoglutarate (α-ketoglutarate) dehydrogenase, and the NAD^+-linked isocitrate dehydrogenase (Hansford and Castro 1985; Denton and McCormack 1990). Activation of CaDHs in the TCA cycle results in increased NADH production, which is critical for matching energy supply with demand during increased workload. NADH is the electron donor of the respiratory chain, and when the respiration rate increases, NADH levels decrease, requiring a concomitant increase in dehydrogenase activity to maintain NADH/NAD^+ redox potential and ATP production. Mitochondria take up $[Ca^{2+}]_c$ through the mitochondrial Ca^{2+} uniporter (mCU) and extrude Ca^{2+} through the mitochondrial Na^+/Ca^{2+} exchanger (mNCE). Accumulating evidence indicates that mitochondria take up $[Ca^{2+}]_c$ during EC coupling, but the kinetics of $[Ca^{2+}]_m$ uptake are still a matter of debate.

In this minireview, we will briefly summarize our current understanding of $[Ca^{2+}]_m$ handling, and discuss the possible effects of $[Ca^{2+}]_m$ on mitochondrial energetics and redox balance in normal and diseased hearts.

Mitochondrial Ca^{2+} uptake

Understanding the kinetics of $[Ca^{2+}]_m$ transport is important to answer how $[Ca^{2+}]_m$ influences energy metabolism. This topic has been debated in terms of whether mitochondrial Ca^{2+} uptake can occur rapidly or results from the slow integration of small increments of Ca^{2+} over many heartbeats (recently discussed by O'Rourke and Blatter 2009). The controversy arises because the kinetics and Km of Ca^{2+} uptake in isolated mitochondria differ from those recorded for $[Ca^{2+}]_m$ changes in some intact cell studies.

Ca^{2+} enters mitochondria via mCU, driven by the large electrochemical gradient for Ca^{2+} across the inner membrane, but this protein has remained undefined after intensive investigation since the 1970s (Saris and Carafoli 2005). Studies of isolated mitochondria estimated the maximal velocity (Vmax) of Ca^{2+} influx via mCU to be approximately $2x10^4$ Ca^{2+} s^{-1} per single mCU molecule, in the range of a fast gated pore (Gunter and Pfeiffer 1990; Gunter et al. 1994). A recent patch clamp study of intact mitoplasts indicated that mCU possesses high Ca^{2+} selectivity with a Ca^{2+}-binding K_d of ~2nM, and the Vmax of Ca^{2+} influx measured in this study was much higher than those in studies of isolated mitochondria ($V_{max}=5\times10^6$ Ca^{2+} s^{-1} per single mCU molecule), as was the channel density (~10–40 channels per μm^2) (Kirichok et al. 2004). The difference between Vmax in the study by Kirichok and in previous studies is likely due to the dissipation of mitochondrial membrane potential, the driving force for mCU-mediated Ca^{2+} influx, by Ca^{2+} influx in previous studies whereas mitochondrial membrane potential was maintained by voltage-clamp in the study by Kirichok (Kirichok et al. 2004).

Although the Vmax estimated for mCU was high, it was argued that rapid uptake of $[Ca^{2+}]_m$ may not be allowed in intact cardiac myocytes due to the low affinity of mCU for Ca^{2+} transport. The Ca^{2+} concentration for half-Vmax of mCU was estimated as ~10–20 μM in studies of isolated mitochondria, which far exceeds the cytosolic bulk Ca^{2+} (1–3 μM). However, rapid $[Ca^{2+}]_m$ uptake is still possible when SR Ca^{2+} release during excitation-contraction coupling (EC coupling) is spatially and temporally limited, which is supported by the concept of a mitochondrial Ca^{2+} microdomain. According to this concept, the sites of Ca^{2+} release are generally located in the space between two mitochondria and spikes of Ca^{2+} are released into a narrow space less than 40 nm away from the nearest mitochondrial membrane, leading to exposure of mCUs to very high concentration of Ca^{2+}, which allows rapid $[Ca^{2+}]_m$ uptake.

A number of studies have been performed to measure $[Ca^{2+}]_m$ in intact cardiomyocytes (review in Maack and O'Rourke 2007; O'Rourke and Blatter 2009). The most common strategy to assess $[Ca^{2+}]_m$ is to load the mitochondrial compartment with a fluorescent Ca^{2+} indicator. The technical challenge for studies using a fluorescent dye is to exclude contamination of $[Ca^{2+}]_m$ signal from cytosolic dye. $[Ca^{2+}]_m$ was also measured by some groups with electron probe microanalysis, which measures total $[Ca^{2+}]_m$ after flash-freezing cells or tissues at different time

points (see refs within Maack and O'Rourke 2007). The failure to observe rapid $[Ca^{2+}]_m$ uptake in some experimental systems needs to be re-examined in light of recent studies demonstrating that variations in the basal physiological conditions, such as the cytosolic Ca^{2+} level or the phosphorylation state, may have an impact on the outcome. Important factors that could influence $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$ handling as well as mitochondrial metabolism, need to be taken into account in order to observe fast $[Ca^{2+}]_m$ transients. For instance, in studies using Mn^{2+} to quench cytosolic fluorescent Ca^{2+} indicators, the inhibitory effect of Mn^{2+} on mCU and oxidative phosphorylation could not be excluded, even though a low level of Mn^{2+} was used and no effect on EC coupling was detected. Similarly, artificial extramitochondrial Ca^{2+} transients applied to partially permeablized cells may ignore spatio-temporal effects present in the native microdomain (Zhou et al. 1998; Sedova et al. 2006). On the other hand, studies demonstrating $[Ca^{2+}]_m$ transients on a beat-to-beat basis were mainly challenged using the argument that mitochondrial fluorescence signals reflect the response of contaminating cytosolic dye. However, careful design of the experiments and the use of novel techniques have produced much more convincing data recently, supporting rapid $[Ca^{2+}]_m$ uptake. In the recent work of our group, we developed a technique (modified from the approach of Zhou et al.) which, with careful attention to eliminate cytosolic signals, allowed us to track $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$ simultaneously with two fluorescent dyes (Maack et al. 2006). Analysis of both $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$ transients recorded with this technique demonstrated a difference in kinetics of the Ca^{2+} transient in two compartments. Moreover, application of inhibitors of mCU and mitochondrial Na^+/Ca^{2+} exchanger (mNCE) showed directionally opposite effects on $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$ transients indicating that fluorescent dyes are localized as expected (Maack et al. 2006). Our results are consistent with studies using genetic targeting techniques to specifically express the luminescent Ca^{2+} sensor aequorin, or FRET-based fluorescent Ca^{2+} sensors, in mitochondria, demonstrating rapid mitochondrial Ca^{2+} uptake on a beat-to-beat basis (Robert et al. 2001; Bell et al. 2006).

In addition to the importance of mitochondrial Ca^{2+} uptake to stimulate oxidative phosphorylation, the mechanism of rapid Ca^{2+} uptake makes mitochondria a relevant buffering system for $[Ca^{2+}]_c$, which influences EC coupling by affecting the $[Ca^{2+}]_c$ transient and may suppress the aberrant propagation of Ca^{2+} sparks.

Mitochondrial Ca^{2+} efflux

There are 2 mechanisms for $[Ca^{2+}]_m$ efflux that have been found so far: Na^+-dependent and Na^+-independent pathways (Puskin et al. 1976; Crompton and Heid 1978). The distribution of these two mechanisms is tissue specific (reviewed by Gunter et al. 2004), and the Na^+-dependent pathway plays a dominant role in Ca^{2+} efflux of cardiac mitochondria (Gunter and Pfeiffer 1990), while the Na^+-independent pathway plays little role (Crompton and Heid 1978; Rosier et al. 1981). The Na^+-dependent pathway acts as an antiporter, extruding mitochondrial 1 Ca^{2+} in exchange of 3 Na^+, which makes this Na^+/Ca^{2+} exchanger (mNCE) an electrogenic transporter (Pfeiffer et al. 2001). The $[Na^+]_i$ dependence of mNCE is sigmoidal with half-maximal velocity ($K_{0.5}$) at ~5–10 mM, which covers the range of physiological $[Na^+]_i$ in the heart (Gunter and Pfeiffer 1990; Cox and Matlib 1993; Bers et al. 2003; Saotome et al. 2005). This makes it possible for intracellular Na^+ to be a critical regulator of mNCE activity under pathophysiological conditions.

Previous studies have shown that mNCE plays a key role in $[Ca^{2+}]_m$ dynamics. $[Ca^{2+}]_m$ efflux is relatively slow when compared to the fast $[Ca^{2+}]_m$ uptake and to the cytosolic Ca^{2+} transient decay rate (Maack et al. 2006; Liu and O'Rourke 2008). At rest, mNCE balances $[Ca^{2+}]_m$ efflux with influx, but when the amplitude and frequency of $[Ca^{2+}]_m$ uptake is increased, the slower extrusion rate will lead to Ca^{2+} accumulation in the matrix: this was shown to be critical for the maintenance of NADH redox potential during increased work. Elevated $[Na^+]_i$ can inhibit $[Ca^{2+}]_m$ accumulation by stimulating mNCE.

Mitochondrial Ca^{2+} and energy metabolism

To be a signal that coordinates energy demand with supply, Ca^{2+} needs to be able to control mitochondrial energy metabolism. Indeed, evidence suggests that Ca^{2+} activates CaDHs of the TCA cycle (Denton and McCormack 1990; Balaban 2002), F_1F_0 ATPase (Yamada and Huzel 1988; Territo et al. 2000), and ANT (Moreno-Sanchez 1985).

Three key enzymes of the TCA cycle are well known to be Ca^{2+} sensitive: pyruvate dehydrogenase, 2-oxoglutarate dehydrogenase and NAD^+-linked isocitrate dehydrogenase (Hansford and Castro 1985; Denton and McCormack 1990), and the $K_{0.5}$ for Ca^{2+} activation of these CaDHs is in the range of 0.7–1 μM (McCormack et al. 1990; Hansford 1991). Activation of these CaDHs by Ca^{2+} increases NADH production, which is the primary electron donor of the electron transport chain. NADH/NAD^+ potential is the driving force of oxidative phosphorylation and an increase of NADH/NAD^+ potential leads to a linear increase of maximal respiration rate in isolated heart mitochondria (Moreno-Sanchez 1985; Mootha et al. 1997).

NADH level is usually considered as an indirect indicator of energetic status of mitochondria, and the

response of NADH to changes in workload was characterized by Brandes and Bers using preloaded cardiac trabeculae (Brandes and Bers 2002). In a state where energy supply and demand are perfectly matched, NADH is maintained at a stable level. When workload is suddenly increased, ATP consumption increases and, according to the feedback mechanism, respiration rate increases, consuming more NADH; consequently, NADH levels should abruptly decrease. However, in most situations, the NADH level recovers quickly, in order to prevent ATP depletion which could cause cell damage, inhibit muscle function, or induce necrotic or apoptotic cell death. The study of Brandes and Bers demonstrated that the recovery of NADH was temporally correlated with rise in $[Ca^{2+}]_m$ after rapid increase in workload (Brandes and Bers 2002), although the fast and slow components of the $[Ca^{2+}]_m$ signal could not be assessed due to overlapping cytosolic Ca^{2+} signals. Studies on the response of NADH to changes in workload in isolated myocytes have produced mixed results (White and Wittenberg 1995; Griffiths et al. 1997; Jo et al. 2006), perhaps due to lack of control over workload and the diversity of cell energy state introduced by cell isolation. Nevertheless, our recent work indicated that $[Ca^{2+}]_m$ accumulation is indispensible for maintaining NADH level during increased work.

In our recent studies, we examined the role of $[Ca^{2+}]_m$ accumulation in matching energy supply and demand by manipulating mitochondrial Ca^{2+} handling using isolated guinea pig cardiac myocytes (Maack et al. 2006; Liu and O'Rourke 2008). In normal conditions, NADH can be maintained upon increased workload with an associated increase of $[Ca^{2+}]_m$. Either Ru360, an mCU blocker, or an increased $[Na^+]_i$, which promotes Ca^{2+} efflux via mNCE, blunted $[Ca^{2+}]_m$ accumulation, resulting in a net oxidation of NADH upon increased workload. Inhibition of the mNCE with CGP-37157 (or raising P_i) enhanced $[Ca^{2+}]_m$ accumulation during increased workload and prevented the oxidation of NADH at increased $[Na^+]_i$. Plotting the NADH level at the end of the stimulation period as a function of $[Ca^{2+}]_m$ during increased workload revealed a linear relationship when $[Ca^{2+}]_m$ accumulation fell below a well-defined threshold, while above the threshold level of $[Ca^{2+}]_m$, NADH remained constant.

Changes in $[Ca^{2+}]_m$ are also likely to influence oxidative phosphorylation at targets besides the CaDHs. Activation of F1/FO ATPase by Ca^{2+} was first suggested in studies of sonicated cardiomyocytes and submitochondrial particles, in which Ca^{2+} increased ATP hydrolytic capacity of the ATPase in the absence of mitochondrial membrane potential ($\Delta\Psi_m$) (Harris and Das 1991). More recently, the direct effect of Ca^{2+} on the ATP synthetic capacity of F1/FO ATPase was demonstrated by Territo (Territo et al. 2000). Their study showed that, when the effects of Ca^{2+} on CaDH and ANT were minimized, Ca^{2+} can still activate oxidative phosphorylation in isolated heart mitochondria with a half-maximal effect of Ca^{2+} at 157 nM (Territo et al. 2000). However, the mechanism of this effect is incompletely understood. Other targets of Ca^{2+} that could potentially affect cardiac energetics include Ca^{2+} activation of ANT (Moreno-Sanchez 1985) and Ca^{2+} modulation of cytochrome c oxidase activity (Bender and Kadenbach 2000).

Effect of mitochondrial Ca^{2+} on redox balance of the cell

As discussed above, Ca^{2+} accumulation is essential for mitochondria to regulate respiration rate by maintaining high $NADH/NAD^+$ redox potential during increased cardiac workload. Recent data from our group (Liu et al. 2009) and others (Knopp et al. 2009) indicates that a sustained net oxidation of the NADH pool is associated with increased production of reactive oxygen species (ROS), suggesting a role for NADH in antioxidant defenses. This can occur because of the close relationship between intermediary metabolism and NADPH, the primary reductant that maintains the functionality of the antioxidant systems of the cell (O'Rourke and Maack 2007)

Mitochondria not only produce ATP, but are also a major source (approximately 90%) of ROS. It has been well documented and widely recognized that ROS have a double role in biological systems as both beneficial signaling molecules and deleterious factors. At low or moderate levels, ROS are involved in redox signaling from mitochondria to the rest of the cell and they play an important role in the regulation of cell function (Droge 2002). Such positive effects are overwhelmed by deleterious effects at high ROS levels or when the antioxidant defenses are compromised. Overproduction of ROS, by causing DNA damage and protein modification, induces cell toxicity and disease pathogenesis (Valko et al. 2007), and, in various cardiac pathologies, an increase of ROS has been identified as a primary or aggravating factor (reviewed by Giordano 2005). Therefore, it is critical for the cell to maintain a low ROS level.

Cells maintain complex redox chains to scavenge ROS and these are ultimately linked to mitochondrial redox balance under normal physiological conditions. The contribution of NADH is manifested through the glutathione (GSH)—dependent and NADPH-dependent pathways. GSH is the most abundant intracellular thiol, and plays an essential role in antioxidant defenses by detoxifying H_2O_2 via the activity of GSH peroxidase which is, in turn, supported by the regeneration of GSH through glutathione reductase, utilizing the redox potential of NADPH. NADPH also is required to maintain the thioredoxin (Trx

$(SH)_2$) and peroxiredoxin pools reduced. These reactive thiol pools, GSH/GSSG and Trx(SH)2/TrxSS, are kept in the reduced state in the matrix by at least three main reactions, i) the NADH/NADPH transhydrogenase, ii) the malic enzyme, and iii) the $NADP^+$-linked isocitrate dehydrogenase reaction (Vogel et al. 1999; Nicholls 2002). The reduction of $NADP^+$ by transhydrogenase depends on NADH and the mitochondrial protonmotive force, while the latter two reactions depend on the levels of TCA cycle intermediates.

Taken together, by increasing NADH production, $[Ca^{2+}]_m$ accumulation contributes to maintaining the driving force for $NADP^+$ reduction and, thereby maintains the redox balance during increased cardiac workload, while a deficiency of Ca^{2+} accumulation, for example induced by Ru360 or elevated $[Na^+]_i$, leads to NADH net oxidation, redox imbalance, and consequently increased ROS production.

Effect of Na^+ on mitochondrial energetics and potential clinical implications

Studies on human cardiac muscles and animal models of cardiac hypertrophy and heart failure indicate that $[Na^+]_i$ is elevated from normal resting levels of 5–8 mM up to 10–22 mM (Pieske and Houser 2003; Pogwizd et al. 2003; Verdonck et al. 2003). Considering that the Km of mNCE for Na^+ is ~5–10 mM, mNCE activity is very sensitive to the change in $[Na^+]_i$ from physiological to pathological levels. The effects of Na^+ on $[Ca^{2+}]_m$ handling and energetics has been previously investigated in isolated heart mitochondria (Cox and Matlib 1993; Babsky et al. 2001). These studies have shown that an increase of extramitochondrial Na^+ leads to decreased $[Ca^{2+}]_m$ levels and compromises mitochondrial energetics, including oxidation of the NADH pool, slowed oxidative phosphorylation rates, and decreased ATP level (Cox and Matlib 1993; Babsky et al. 2001). Babsky et al. (2001) also revealed that heart mitochondria isolated from diabetic animals, in which cytoplasmic Na^+ is chronically elevated, had lower basal ATP levels compared to control animals (Babsky et al. 2001). With respect to chronic cardiac disease models, we recently demonstrated that elevated $[Na^+]_i$ in myocytes from failing cells blunted $[Ca^{2+}]_m$ accumulation and caused net oxidation of the NADH pool (Maack et al. 2006; Liu and O'Rourke 2008), as well as increased ROS production (Liu et al. 2009).

All of these findings indicate that increased $[Na^+]_i$ may compromise cardiac function in several ways. First, blunted $[Ca^{2+}]_m$ loading could impair the local buffering capacity of mitochondria near the dyad, which could contribute to uncontrolled spontaneous Ca^{2+} wave propagation, as previously reported (Mackenzie et al. 2004; Seguchi et al. 2005). Second, decreased $NADH/NAD^+$ redox potential could lead to a mismatch in energy supply and demand. Third, the net oxidation of the NAD(P)H pool could affect the antioxidant scavenging systems of the cell. All of these actions could contribute to premature cell death in the context of chronic heart disease, particularly in the presence of a sustained work overload condition, impaired Ca^{2+} handling, and extrinsic and intrinsic sources of oxidative stress. The ability of the mNCE inhibitor to prevent the oxidation of NAD(P)H pool demonstrated in our recent work suggests a remedy for the deleterious effects of increased $[Na^+]_i$ in the context of heart failure.

Elucidation of the both the feedforward and feedback effects of Ca^{2+}, Na^+ and NAD(P)H redox balance on excitation-contraction-energetic coupling in normal and diseased hearts represents a fertile area of investigation in the future.

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