Calcium release microdomains and mitochondria

Michael Kohlhaas and Christoph Maack*

Klinik für Innere Medizin III, Universitätsklinikum des Saarlandes, Homburg 66421, Germany

Received 12 December 2012; revised 6 February 2013; accepted 6 February 2013; online publish-ahead-of-print 14 February 2013

Abstract

The processes of excitation–contraction (EC) coupling consume large amounts of energy that need to be replenished by oxidative phosphorylation in the mitochondria. Since Ca\textsuperscript{2+} activates key enzymes of the Krebs cycle in the mitochondrial matrix, it is important to understand the mechanisms and kinetics of mitochondrial Ca\textsuperscript{2+} uptake to delineate how in cardiac myocytes, energy supply is efficiently matched to demand. In recent years, the identification of various proteins involved in mitochondrial Ca\textsuperscript{2+} signalling and the tethering of mitochondria to the sarcoplasmic reticulum (SR) has considerably advanced the field and supported the concept of a mitochondrial Ca\textsuperscript{2+} microdomain, in which Ca\textsuperscript{2+} concentrations are high enough to overcome the low Ca\textsuperscript{2+} affinity of the principal mitochondrial Ca\textsuperscript{2+} uptake mechanism, the Ca\textsuperscript{2+} uniporter. Furthermore, defects in EC coupling that occur in heart failure disrupt SR-mitochondrial Ca\textsuperscript{2+} crosstalk and may cause energetic deficit and oxidative stress, both factors that are thought to be causally involved in the initiation and progression of the disease.

Keywords

Calcium • Mitochondria • Microdomain • Redox • Heart failure

The article is part of the Spotlight Issue on: T-tubules and ryanodine receptor microdomain signalling in cardiac hypertrophy and failure.

1. Introduction

Excitation–contraction (EC) coupling in cardiac myocytes consumes vast amounts of energy in the form of ATP that need to be efficiently replenished by oxidative phosphorylation in the mitochondria, which occupy roughly a third of cell volume. During a single heartbeat, ~2% of the cellular ATP is consumed, and the whole ATP pool of cardiac myocytes is turned over within less than a minute.\textsuperscript{1–3} To orchestrate oxidative phosphorylation in response to constantly changing workloads of the heart, various factors tightly control the mitochondrial redox state and electron flux along the respiratory chain, securing constant availability of ATP. Two of the most important regulatory factors in this regard are Ca\textsuperscript{2+} and ADP\textsuperscript{4–7} whose communication between different organelles and/or compartments within cardiac myocytes is organized by ‘microdomains’.\textsuperscript{8,9} Spatial regulation by microdomains is a particular requirement for signalling efficacy considering the highly organized architecture of cardiac myocytes, in which mitochondria comprise a third of the cell volume and are aligned regularly along the ATP-consuming myofilaments, tightly connected to the Ca\textsuperscript{2+} stores (i.e. the sarcoplasmic reticulum, SR) and strategically positioned in vicinity to the ‘dyads’, where activation of L-type Ca\textsuperscript{2+} channels (LTCC) triggers Ca\textsuperscript{2+} release from the junctional SR in a process coined ‘Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release’.

In the last couple of years, considerable progress has been made regarding the identification of the molecular nature and functional roles of various components involved in the transmission of Ca\textsuperscript{2+} between the SR and mitochondria. In particular, the identification of the molecular identities and functional roles of various proteins in the inner mitochondrial membrane (IMM) and outer mitochondrial membrane (OMM), but also the SR, has paved new avenues and spurred new enthusiasm in research on the mechanisms of mitochondrial Ca\textsuperscript{2+} uptake. Here, after giving a brief overview on EC coupling and mitochondrial energetics (for a more comprehensive review, see refs.\textsuperscript{5,10}), we focus on these more recent findings with a special emphasis on the aspects relevant to cardiac myocytes and the pathophysiological changes that occur in chronic heart failure.

2. Excitation–contraction coupling

2.1 Physiology

During a cardiac action potential (AP), Ca\textsuperscript{2+} enters cardiac myocytes via LTCC, triggering an even greater release of Ca\textsuperscript{2+} from the SR via ryanodine receptors (RyR2), and this Ca\textsuperscript{2+} is available at the myofilaments to induce contraction. The amount of Ca\textsuperscript{2+} that entered the cell via LTCC is exported primarily via the sarcolemmal Na\textsuperscript{+}/Ca\textsuperscript{2+}-...
exchanger (NCX) during diastole, whereas the Ca\(^{2+}\) that was released from the SR is taken back up by the SR Ca\(^{2+}\) ATPase (SERCA). In human, rabbit and guinea-pig cardiac myocytes, the distribution between SR-derived Ca\(^{2+}\) and LTCC-triggered Ca\(^{2+}\) influx is ~70/30, whereas in smaller animals (such as mice and rats), this is shifted towards an even higher contribution of the SR (~90/10).\(^8\) The interplay between LTCC and RyR2 is facilitated by the close vicinity of both channels in the dyads between t-tubular sarclemma and the junctional SR. The concept of such a Ca\(^{2+}\) microdomain is that for a brief period, the Ca\(^{2+}\) concentrations at the site of release (i.e. near the RyR2 or the LTCC) exceed the concentrations in the bulk cytosol of the cell by several orders of magnitude.\(^8\)\(^,\)\(^10\) These Ca\(^{2+}\) peaks are limited spatially and temporarily by diffusion of Ca\(^{2+}\) away from these ‘hot spots’ to the rest of the cytosol, where Ca\(^{2+}\) binds to myofilaments to induce contraction. A similar microdomain exists between the SR and mitochondria (Figure 1 with inset), facilitating an efficient Ca\(^{2+}\) transfer between both organelles to match the energy produced in the mitochondria to the demand generated by Ca\(^{2+}\)-dependent processes of EC coupling. We will allude to this mitochondrial Ca\(^{2+}\) microdomain—the focus of this review—in more detail further below.

2.2 Pathophysiology

In chronic heart failure, defects of EC coupling underlie systolic and diastolic dysfunction.\(^5\)\(^,\)\(^12\)\(^,\)\(^13\) A central defect in failing cardiomyocytes is the decreased Ca\(^{2+}\) load of the SR, which is related to decreased SERCA activity and a leak of the RyR2. Furthermore, the three-dimensional t-tubular structure that provides efficient coupling of the membrane potential to trigger SR Ca\(^{2+}\) release is disturbed in various models of heart failure, which induces spatio-temporal dyssynchrony of cytosolic Ca\(^{2+}\) (Figure 1)\(^,\)\(^15\)\(^,\)\(^16\) Initially considered as an adaptive mechanism, the cytosolic Na\(^+\) concentration ([Na\(^+\)]) is elevated in failing cardiomyocytes, presumably related to an activation of the ‘late Na\(^+\) current’ (late \(i_{	ext{Na}}\)) and/or increased activity of the Na\(^+\)/H\(^+\) exchanger.\(^17\)\(^–\)\(^21\) This elevation of [Na\(^+\)], in particular in combination with up-regulation of the NCX protein, favours the reverse-mode of the NCX to import Ca\(^{2+}\) during the AP and to decrease Ca\(^{2+}\)-efflux during diastole.\(^17\)\(^,\)\(^19\)\(^,\)\(^22\)\(^–\)\(^25\) Thus, it is generally perceived that this elevation in [Na\(^+\)], partly compensates for decreased SR Ca\(^{2+}\) load and improves contractility in heart failure.\(^12\)\(^,\)\(^22\)\(^–\)\(^27\) In this review, we will also discuss how the elevation of [Na\(^+\)], has a negative impact on mitochondrial Ca\(^{2+}\) uptake and redox state.

A principal mechanism to regulate EC coupling is \(\beta\)-adrenergic stimulation, which signals through phosphorylation of Ca\(^{2+}\) handling proteins mediated by protein kinase A (PKA) and Ca\(^{2+}\)/Calmodulin-dependent protein kinase II (CaMKII).\(^23\)\(^,\)\(^24\) In heart failure, chronic \(\beta\)-adrenergic stimulation with subsequent dysregulation of CaMKII- and PKA-regulated phosphorylation of target proteins substantially contributes to the maladaptive changes of EC coupling (e.g. the leak of RyR2 and activation of late \(i_{	ext{Na}}\)).\(^28\)\(^–\)\(^30\) Furthermore, an energetic deficit\(^31\)\(^,\)\(^32\) and mitochondrial oxidative stress\(^33\)\(^,\)\(^34\) are thought to play causative roles in the progression of heart failure. Over the past couple of years, we developed the concept that based on the tight interplay between EC coupling, mitochondrial redox state, and the regulation of reactive oxygen species (ROS) production, defects in EC coupling play a causal role for the development of energetic deficit and oxidative stress in heart failure (Figure 1).\(^35\)\(^,\)\(^35\)

### 3. Regulation of mitochondrial energetics and redox state during EC coupling

3.1 Matching energy supply and demand

In the mitochondrial matrix, the Krebs cycle produces NADH and FADH\(_2\), which fuel electrons into the electron transport chain (ETC) through complexes I and II (Figure 1). This induces sequential redox reactions along the complexes of the ETC that promote the translocation of protons (H\(^+\)) across the IMM, creating a proton gradient (\(\Delta\text{pH}\)) and an electrical gradient (\(\Delta\text{YM}\)) which constitute the proton motive force (\(\Delta\mu\text{H}^+\)) which is utilized by the \(F_\text{F}_\text{O}_\text{ADP}\)–ATP synthase to regenerate ATP from ADP. In vivo, cardiac workload is increased especially by \(\beta\)-adrenergic stimulation, increasing the rate and amplitude of cytosolic Ca\(^{2+}\) transients, which in turn accelerates ATP consumption by Ca\(^{2+}\)-cycling proteins and the myofilaments. This pronounced ATP hydrolysis elevates the formation of ADP, stimulating ATP regeneration at the \(F_\text{F}_\text{O}_\text{ADP}\)–ATP synthase. Since the \(F_\text{F}_\text{O}_\text{ADP}\)–ATP synthase reaction is coupled to \(\Delta\mu\text{H}^+\), ATP production principally dissipates \(\Delta\mu\text{H}^+\). To maintain \(\Delta\mu\text{H}^+\), NADH donates more electrons to the ETC, which oxidizes NADH to NAD\(^+\) (and FADH\(_2\) to FAD). Since \(\beta\)-adrenergic stimulation elevates the rate and frequency of cytosolic Ca\(^{2+}\) transients, mitochondria accumulate Ca\(^{2+}\) through several uptake mechanisms,\(^36\)\(^,\)\(^37\) but in particular, the mitochondrial Ca\(^{2+}\) uniporter (MCU; Figure 1).\(^38\)\(^,\)\(^39\) In the mitochondrial matrix, Ca\(^{2+}\) stimulates key enzymes of the Krebs cycle to accelerate the regeneration of oxidized NAD\(^+\) and FAD to reduced NADH and FADH\(_2\).\(^4\)\(^,\)\(^40\)\(^–\)\(^46\) Furthermore, Ca\(^{2+}\)-induced stimulation of the ETC, the \(F_\text{F}_\text{O}_\text{ADP}\)–ATP synthase and the aspartate–glutamate shuttle further contribute to an acceleration of oxidative phosphorylation (for a recent review, see Glancy and Balaban\(^47\)). Thus, Ca\(^{2+}\) \& ADP regulate oxidative phosphorylation in a complimentary manner and secure relatively constant ratios of ATP/ADP,\(^48\)\(^,\)\(^49\) and constant or even increasing ratios of NADH/NAD\(^+\) during physiological variations of cardiac workload.

This complex interplay between ADP- and Ca\(^{2+}\)-induced regulation of oxidative phosphorylation and the mitochondrial redox state has been revealed by experiments in which abrupt changes of workload were provoked in isolated cardiac trabeculae,\(^4\)\(^0\)\(^–\)\(^4\)\(^2\) cardiac myocytes,\(^4\)\(^3\)\(^,\)\(^4\)\(^4\) and/or computational modelling.\(^4\)\(^5\)\(^,\)\(^4\)\(^6\) During more gradual escalations of workload, as they occur during \(\beta\)-adrenergic stimulation in vivo, these transitions of energetic intermediates are presumably less accentuated, reflecting the high efficiency of regulatory control mechanisms (i.e. Ca\(^{2+}\) \& ADP) that quickly adapt the supply to the increased energy demand. Thus, given the central role of Ca\(^{2+}\) for regulating mitochondrial energetics, the mechanisms and kinetics of mitochondrial Ca\(^{2+}\) uptake are of utmost importance for our understanding of energy supply-and-demand matching in the heart.

### 3.2 Role of redox state for mitochondrial reactive oxygen species formation

In addition to its role for energy production, mitochondrial Ca\(^{2+}\) uptake also plays a key role in governing mitochondrial formation of ROS. It has been initially proposed that ~2% of O\(_2\) consumption is aberrantly funneled to superoxide (O\(_2^-\)) production, however, these initial estimates were corrected to levels of ~0.2% more recently (for review, see Balaban et al\(^5\)\(^1\)). The major site of O\(_2^-\) formation are complexes I and III of the ETC (Figure 1).\(^5\)\(^1\)\(^,\)\(^2\) and
Figure 1: Mitochondrial Ca$^{2+}$ signalling and its impact on mitochondrial energetics and redox state in health and disease. The Krebs cycle regenerates NADH required for oxidative phosphorylation at the electron transport chain, but also NADPH through isocitrate dehydrogenase (IDP_m), malic enzyme (MEP) and nicotinamide nucleotide transhydrogenase (Nnt). Ca$^{2+}$ stimulates three key enzymes of the Krebs cycle and thus, regulates NADH and NADPH regeneration. In heart failure, defects in EC coupling and mitochondrial ion homeostasis (indicated by red arrows) contribute to energetic mismatch and oxidative stress. Δ$\Psi_{m}$, mitochondrial membrane potential; Mn-SOD, Mn$^{2+}$-dependent superoxide dismutase; PRX, peroxiredoxin; GPX, glutathione peroxidase; TRX, reduced/oxidized thioredoxin; GTX, thioredoxin reductase; GR, glutathione reductase; α-KG, α-ketoglutarate; MCU, mitochondrial Ca$^{2+}$ uniporter; NCLX, mitochondrial Na$^{+}$/Ca$^{2+}$ (and Li$^{+}$) exchanger; $p_{o}$, open probability; RyR, ryanodine receptor; SERCA, SR, Ca$^{2+}$ ATPase; Mfn, mitofusin. Inset: detailed aspects of a mitochondrial Ca$^{2+}$ microdomain. MICU1, mitochondrial Ca$^{2+}$ uptake 1; mNHE, mitochondrial Na$^{+}$/H$^{+}$ exchanger; IMM and OMM, inner and outer mitochondrial membranes; VDAC, voltage-dependent anion channel.
O$_2$—formation is highest when substrates (glucose, fatty acids) are available, but energy (and thus, O$_2$) consumption are low, i.e. a condition that is experimentally defined as ‘state 4 respiration’. In this situation, which typically does not occur in the heart where energy is constantly consumed, the ETC is highly reduced, and electrons are more likely to aberrantly ‘slip’ to O$_2$ to produce O$_2^-$.

When workload increases, ADP-induced acceleration of respiration oxidizes the ETC by favouring the enzymatic reduction of O$_2$ to H$_2$O at complex IV (cytochrome oxidase), during which O$_2^-$ formation is efficiently avoided. At the same time, aberrant O$_2^-$ formation at complexes I and III is diminished.

Besides the ETC, other mechanisms of mitochondrial ROS production have been proposed. Dedkova and Blatter revealed that an uncoupled nNOS located in the mitochondria is a source for O$_2^-$ which can induce mPTP opening and cell death under pathological conditions of mitochondrial Ca$_{\text{2+}}$ overload. The existence of a mitochondrial NOS, however, is still controversial (for review, see Brookes).

Furthermore, translocation of MAO and NAPDH oxidase 4 (Nox4) to mitochondria may contribute to mitochondrial oxidative stress. The respective contribution of these various potential sources to overall ROS production, however, requires further evaluation.

To protect the matrix enzymes and mitochondrial DNA from oxidative damage, antioxidative mechanisms are in place to eliminate ROS. O$_2^-$ is rapidly transformed to H$_2$O$_2$ by superoxide dismutase (SOD), and H$_2$O$_2$ is detoxified by glutathione peroxidase (GPX) and peroxiredoxin (PRX; Figure 1). These H$_2$O$_2$-eliminating enzymes need to be regenerated by NADPH-dependent enzymes, such as glutathione reductase (GR) and thioredoxin reductase (TR). This implies a redox feedback on EC coupling.

Mitochondria take up Ca$_{\text{2+}}$ primarily via the MCU, a recently identified 40-kDa channel protein in the IMM that is highly selective for Ca$_{\text{2+}}$ (Figure 1). In COS7 cells, the MCU has a high Ca$_{\text{2+}}$ carrying capacity (5 × 10$^8$ Ca$_{\text{2+}}$ molecules per second per single MCU molecule), and MCU density in the IMM (∼10–40 pm$^2$) is only slightly lower than that of voltage-gated Ca$_{\text{2+}}$ channels in the sarcolemmal membrane (∼100 pm$^2$). More recent evidence, however, showed that mouse heart mitochondria have dramatically lower MCU current densities than skeletal muscle mitochondria. It is currently unclear whether this low current density is limited to the mouse with heart rates of ∼600/min to avoid mitochondrial Ca$_{\text{2+}}$ overload, or conserved across many species. The driving force for mitochondrial Ca$_{\text{2+}}$ uptake is the large electrochemical gradient across the IMM (ΔΨ$_{\text{mm}}$ ∼ −180 mV), and early experiments on suspensions of isolated mitochondria revealed Ca$_{\text{2+}}$ concentrations for half-maximal activation (K$_{\text{50}}$) of the MCU in the upper micromolar range.

In more recent patch-clamp-based studies, the K$_{\text{50}}$ was even higher in the millimolar range. These Ca$_{\text{2+}}$ concentrations are clearly beyond levels expected in the bulk cytoplasm during Ca$_{\text{2+}}$ transients in myocytes (∼1–2 μmol/L). In a study on human cardiac mitoplasts, two distinct mitochondrial Ca$_{\text{2+}}$ channels were identified, coined mCa1 and mCa2, with mCa1 resembling the properties of the MCU and mCa2 with slightly different properties, possibly responsible for part of the non-Ru360-sensitive component of mitochondrial Ca$_{\text{2+}}$ uptake. Similarly, Wei et al. identified two different modes of mitochondrial Ca$_{\text{2+}}$ uptake with different sensitivities to Ru360, termed MCU$_{\text{node1}}$ and MCU$_{\text{node2}}$. This will be discussed in more detail below.

The MCU is part of a macromolecular complex that also contains MICU1 (Figure 1, inset), the first protein involved in mitochondrial Ca$_{\text{2+}}$ uptake whose molecular nature could be identified in HeLa cells by the Mootha group. The identity of MICU1 was revealed by an integrative strategy that predicted human genes involved in mitochondrial Ca$_{\text{2+}}$ uptake based on clues from comparative physiology, evolutionary genomics, and organelle proteomics.

**4. Mechanisms and kinetics of mitochondrial Ca$_{\text{2+}}$ uptake and release**

Although in recent years, considerable progress was made with regard to the molecular identity of proteins involved in mitochondrial Ca$_{\text{2+}}$ uptake and release, several questions still remain unanswered and some controversies persist. As already addressed by Hüser et al. in 2000, a central matter of debate was and is whether mitochondria take up Ca$_{\text{2+}}$ rapidly, on a beat-to-beat basis, or rather slowly, integrating changes of amplitudes and frequency of cytosolic Ca$_{\text{2+}}$ transients. This controversy is based partly on different findings related to different techniques that can be applied to measure mitochondrial Ca$_{\text{2+}}$ uptake in cardiac myocytes during EC coupling (for more comprehensive reviews, see refs. 5,71,74). While most groups agree that beat-to-beat mitochondrial Ca$_{\text{2+}}$ uptake can occur, the absolute quantities of mitochondrial Ca$_{\text{2+}}$ influxes during cytosolic Ca$_{\text{2+}}$ transients remain incompletely resolved, ranging from estimates of 10 nMbeat$^{-1}$ to ∼26% of the whole SR Ca$_{\text{2+}}$ content during a cytosolic Ca$_{\text{2+}}$ transient.

**4.1 Kinetics and molecular identity of the mitochondrial Ca$_{\text{2+}}$ uniporter**

Mitochondria take up Ca$_{\text{2+}}$ primarily via the MCU, a recently identified 40-kDa channel protein in the IMM that is highly selective for Ca$_{\text{2+}}$ (Figure 1). In COS7 cells, the MCU has a high Ca$_{\text{2+}}$ carrying capacity (5 × 10$^8$ Ca$_{\text{2+}}$ molecules per second per single MCU molecule), and MCU density in the IMM (∼10–40 pm$^2$) is only slightly lower than that of voltage-gated Ca$_{\text{2+}}$ channels in the sarcolemmal membrane (∼100 pm$^2$). More recent evidence, however, showed that mouse heart mitochondria have dramatically lower MCU current densities than skeletal muscle mitochondria.

It is currently unclear whether this low current density is limited to the mouse with heart rates of ∼600/min to avoid mitochondrial Ca$_{\text{2+}}$ overload, or conserved across many species. The driving force for mitochondrial Ca$_{\text{2+}}$ uptake is the large electrochemical gradient across the IMM (ΔΨ$_{\text{mm}}$ ∼ −180 mV), and early experiments on suspensions of isolated mitochondria revealed Ca$_{\text{2+}}$ concentrations for half-maximal activation (K$_{\text{50}}$) of the MCU in the upper micromolar range.

In more recent patch-clamp-based studies, the K$_{\text{50}}$ was even higher in the millimolar range. These Ca$_{\text{2+}}$ concentrations are clearly beyond levels expected in the bulk cytoplasm during Ca$_{\text{2+}}$ transients in myocytes (∼1–2 μmol/L). In a study on human cardiac mitoplasts, two distinct mitochondrial Ca$_{\text{2+}}$ channels were identified, coined mCa1 and mCa2, with mCa1 resembling the properties of the MCU and mCa2 with slightly different properties, possibly responsible for part of the non-Ru360-sensitive component of mitochondrial Ca$_{\text{2+}}$ uptake. Similarly, Wei et al. identified two different modes of mitochondrial Ca$_{\text{2+}}$ uptake with different sensitivities to Ru360, termed MCU$_{\text{node1}}$ and MCU$_{\text{node2}}$. This will be discussed in more detail below.

The MCU is part of a macromolecular complex that also contains MICU1 (Figure 1, inset), the first protein involved in mitochondrial Ca$_{\text{2+}}$ uptake whose molecular nature could be identified in HeLa cells by the Mootha group. The identity of MICU1 was revealed by an integrative strategy that predicted human genes involved in mitochondrial Ca$_{\text{2+}}$ uptake based on clues from comparative physiology, evolutionary genomics, and organelle proteomics.
Systematic RNA interference experiments against 13 top candidates of this screen revealed MICU1 to be a protein that is associated with the IMM and that has two canonical EF hands that are required for its activity, indicating a role in Ca\(^{2+}\) sensing (Figure 1, inset).\(^{66}\) Silencing MICU1 abolished mitochondrial Ca\(^{2+}\) uptake and attenuated metabolic coupling between cytosolic Ca\(^{2+}\) transients and activation of Krebs cycle dehydrogenases.\(^{66}\) The observation that MICU1 overexpression did not enhance mitochondrial Ca\(^{2+}\) uptake and the fact that MICU1 has only one membrane-spanning domain, however, made it an unlikely candidate for the Ca\(^{2+}\) unipor- ter per se, but rather a regulatory protein that would act as a Ca\(^{2+}\) sensor based on the requirement of its two EF hands for proper function (see below).

In late 2011, two independent seminal papers from the Mootha and the Rizzuto groups unequivocally revealed the molecular identity of the Ca\(^{2+}\) uniporter.\(^{38,39}\) Using comparable systematic computational screening approaches, a protein with two membrane-spanning domains that locates to the IMM was identified, now termed the MCU (Figure 1, inset). In contrast to MICU1, overexpression of MCU doubled mitochondrial Ca\(^{2+}\) uptake in HeLa cells,\(^{38}\) while silencing MCU substantially reduced mitochondrial Ca\(^{2+}\) uptake in HeLa cells in vitro,\(^{38,39}\) but also in liver mitochondria in vivo.\(^{38}\) Reconstitution of the MCU in lipid bilayers yielded channel activity that resembled the electrophysiological properties and inhibitor sensitivity of the uniporter.\(^{23}\) The MCU forms oligomers, physically interacts with MICU1 and is part of a multi-protein complex. According to both studies, a domain, however, made it an unlikely candidate for the Ca\(^{2+}\) uniporter per se, but rather a regulatory protein that would act as a Ca\(^{2+}\) sensor based on the requirement of its two EF hands for proper function (see below).

Recently, the group of Anderson proposed an important regulatory role for CaMKII in regulating MCU activity.\(^{90}\) They observed that CaMKII promoted opening of the mitochondrial PTP and myocardial death by enhancing the MCU current. A mitochondrial-targeted CaMKII-inhibitory protein reduced the MCU current, prevented mPTP opening (presumably by limiting mitochondrial Ca\(^{2+}\) overload after ischaemia/reperfusion), \(\Delta\Phi_m\) dissipation, programmed cell death and infarct size to a similar extent as cyclosporine A, an inhibitor of the PTP.\(^{90}\) Based on patch-clamp measurements, it was proposed that CaMKII regulates the MCU from the matrix side of the IMM.\(^{90}\) Since CaMKII activity is increased by GPCR activation and oxidative stress, CaMKII-mediated regulation of MCU may resemble an attractive control mechanism of mitochondrial Ca\(^{2+}\) uptake. This concept, and in particular its relevance under physiological conditions, will require further investigation.

In addition to the MCU, several other mechanisms and/or channels for mitochondrial Ca\(^{2+}\) uptake have been proposed. Among them are the rapid mode of uptake (RaM), coenzyme Q10, RyR1, uncoupling proteins 2 and 3 and leucine-zipper-EF-hand-containing transmembrane protein 1 (Letm1). For a more comprehensive review on these Ca\(^{2+}\) uptake mechanisms, we refer the reader to two recent comprehensive reviews by O-Uchi et al.\(^{36}\) and Dedkova and Blatter.\(^{37}\)

### 4.2 Mitochondrial Ca\(^{2+}\) efflux

The primary mitochondrial Ca\(^{2+}\) efflux mechanism in cardiac myocytes is the mitochondrial NCX,\(^{81,91}\) which is presumably electrogenic\(^{92,93}\) by exchanging 1 Ca\(^{2+}\) for 3 Na\(^{+}\), with a \(K_m\) for [Na\(^{+}\)] of \(~8\ \text{mM}\) and thus, within the physiological range of [Na\(^{+}\)], in cardiac myocytes.\(^{21,95}\) Recently, the molecular nature of the mitochondrial NCX was identified as well.\(^{64}\) The exchange forms dimers, locates to the mitochondrial cristae, transports either Na\(^{+}\) or Li\(^{+}\) in exchange for Ca\(^{2+}\) and thus, is termed NCLX (Figure 1).\(^{64}\) Ca\(^{2+}\)-dependent mitochondrial Na\(^{+}\) import via the NCLX is counter-balanced by mitochondrial Na\(^{+}\)H\(^{+}\) exchange (mNHE). This makes mitochondrial Ca\(^{2+}\) uptake an energetically expensive process, since \(\Delta\mu_{\text{m}}\) is dissipated by the Na\(^{+}\)-dependent H\(^{+}\) import to the matrix and needs to be regenerated by the ETC (Figure 1).

### 4.3 Integrative physiology of mitochondrial Ca\(^{2+}\) uptake and release in cardiac myocytes

While these studies are important to understand the molecular composition of the MCU with its regulatory proteins in general, they have not (yet) resolved the kinetics and quantities of mitochondrial Ca\(^{2+}\) uptake in cardiac myocytes during EC coupling. In the last couple of years, we have focused on this controversial issue by establishing a method by which cytosolic ([Ca\(^{2+}\)]\(_c\)) and mitochondrial Ca\(^{2+}\) concentrations ([Ca\(^{2+}\)]\(_m\)) are monitored in the same cell, respectively,\(^{43}\) by loading myocytes with a cell-permeable Ca\(^{2+}\) indicator that—due to its positive charge—accumulates primarily in the mitochondrial matrix (the ester form of rhod-2, i.e. rhod-2/AM). To eliminate cytosolic traces of the dye, myocytes are patch-clamped and dialysed with a rhod-2-free pipette solution that contains indo-1 salt that locates only to the cytosol.\(^{53}\) Eliciting cytosolic Ca\(^{2+}\) transients by voltage-clamping, we observed that during \(\beta\)-adrenergic stimulation (a situation when the heart requires increased ATP production), mitochondria take up Ca\(^{2+}\) rapidly, with [Ca\(^{2+}\)]\(_m\) transients peaking slightly earlier than cytosolic Ca\(^{2+}\) transients.\(^{43,44}\) This slightly earlier
[Ca\(^{2+}\)]_m peak could not be explained by different dye kinetics and would be in agreement with the idea that mitochondria are located close to the SR where they would sense the RyR-released Ca\(^{2+}\) earlier (and at higher concentrations) than Ca\(^{2+}\) that is monitored by indo-1 in the bulk cytosol. Furthermore, [Ca\(^{2+}\)]_m transients had 2.5-fold slower decay kinetics than [Ca\(^{2+}\)]_c.\(^{43,44}\) This leads to accumulation of diastolic [Ca\(^{2+}\)]_m during an increase in the amplitude and/or frequency of cytosolic Ca\(^{2+}\) transients. Blocking mitochondrial Ca\(^{2+}\) uptake with Ru360 reduced the mitochondrial Ca\(^{2+}\) transients by ~66%, whereas cytosolic Ca\(^{2+}\) transients slightly increased.\(^{43,44}\) Blocking the primary mitochondrial Ca\(^{2+}\) export mechanism (i.e. the NCLX) increased diastolic [Ca\(^{2+}\)]_m and decreased [Ca\(^{2+}\)]_c transients,\(^{43}\) while elevating [Na\(^+\)] to activate the NCLX had the opposite effect, accelerating mitochondrial Ca\(^{2+}\) efflux, reducing steady-state [Ca\(^{2+}\)]_m while slightly elevating [Ca\(^{2+}\)]_c.\(^{43}\)

Since in our studies, rhod-2 was not calibrated to quantify [Ca\(^{2+}\)]_m, it remained unclear so far how much Ca\(^{2+}\) indeed enters mitochondria during a cytosolic Ca\(^{2+}\) transient. This question had spurred some controversy, since from indirect estimations (see below under ‘Evidence for a mitochondrial microdomain’) it seemed that mitochondria took up more Ca\(^{2+}\) than just 1% of overall cytosolic Ca\(^{2+}\) as originally proposed by the Bers group.\(^{96,97}\) Maack et al.\(^{43}\) and others\(^{85,98}\) observed that when blocking mitochondrial Ca\(^{2+}\) uptake, the amplitude of cytosolic Ca\(^{2+}\) transients increased, although in our more recent studies,\(^{44}\) this effect was smaller than in our initial study and no longer significant.\(^{43}\)

Recently, the Bers group further addressed this issue. After their previous study using rhod-2 in permeabilized rat cardiac myocytes, in which they estimated that 2–10 nM Ca\(^{2+}\) enter mitochondria per beat,\(^{75}\) they now used the mitochondrially targeted inverse pericam indicator Mitycam, which was previously established in cardiac myocytes by the group of Smith,\(^{99}\) to measure mitochondrial Ca\(^{2+}\) uptake in intact rabbit cardiac myocytes.\(^{76}\) With high spatial resolution microscopy, they observed that areas of mitochondria that are located within 500 nm from the dyads (i.e. the Z-line) have an amplitude of [Ca\(^{2+}\)]_m of 37 nM per beat, while areas of mitochondria that are further (>500 nm) from the dyads have increases in [Ca\(^{2+}\)]_m of 26 nM per cytosolic Ca\(^{2+}\) transient. Furthermore, the time-to-peak of [Ca\(^{2+}\)]_m was shorter close to the Z-lines than in more remote areas of mitochondria, and since the MCU was evenly distributed over mitochondria, these data support the idea that spatial aspects (i.e. the existence of a mitochondrial Ca\(^{2+}\) microdomain; see also below) play an important role for the efficiency of mitochondrial Ca\(^{2+}\) uptake. Overall, these measurements support the concept that the quantities of mitochondrial Ca\(^{2+}\) uptake contribute only ~1% to cytosolic Ca\(^{2+}\) handling, which argues against the idea that mitochondrial Ca\(^{2+}\) uptake ‘shapes’ cytosolic Ca\(^{2+}\) transients in cardiac myocytes.

Although this study is presently the best quantitative estimation of mitochondrial Ca\(^{2+}\) uptake during EC coupling in cardiac myocytes, there are still some methodological issues that need to be taken into account when interpreting the data. To express Mitycam in cardiac myocytes, cells had to be cultured for 36 h before the functional experiments,\(^{76}\) which may have an effect on the t-tubular organization of myocytes. Furthermore, the K_g of Mitycam for Ca\(^{2+}\) is ~200 nmol/L,\(^{76,99}\) which may lead to saturation of fluorescence should [Ca\(^{2+}\)]_m approach micromolar concentrations. A potentially important difference between this study\(^{76}\) and our previous studies with rhod-2 AM\(^{43,44,100}\) is that our experiments were carried out at 37°C in freshly isolated cardiac myocytes, and the times to peak (TTP) of cytosolic and mitochondrial Ca\(^{2+}\) transients were <60 ms, respectively\(^{43,44,100}\). In contrast, the study by Lu et al.\(^{76}\) was carried out at room temperature, and TTP of [Ca\(^{2+}\)]_c and [Ca\(^{2+}\)]_m were between 200 and 300 ms. We observed that the rate of increase in [Ca\(^{2+}\)]_c, \(\text{which correlates with SR Ca}^{2+}\text{ release flux}\),\(^{101,102}\) had a strong impact on the efficiency of mitochondrial Ca\(^{2+}\) uptake. In conditions under which the TTP of [Ca\(^{2+}\)]_c was 200–300 ms (i.e. in response to NCX-mediated Ca\(^{2+}\) influx triggering SR Ca\(^{2+}\) release), the efficiency of mitochondrial Ca\(^{2+}\) uptake was only 50% compared with conditions with rapid IC\(_{\text{L}},\text{induced SR Ca}^{2+}\text{ release}^{100}\). Taken together, the study of Lu et al.\(^{76}\) makes a strong and conclusive point that overall mitochondrial Ca\(^{2+}\) fluxes are rather low, but due to the mentioned technical limitations the actual mitochondrial Ca\(^{2+}\) uptake under ‘real life’ conditions may still be slightly underestimated. Nevertheless, the concept is further supported by a recent study that observed that the current of the MCU is dramatically lower in cardiac compared with skeletal muscle mitochondria of the mouse.\(^{80}\)

Another level of complexity to the quantitative aspects of mitochondrial Ca\(^{2+}\) uptake and free matrix concentrations is added by the largely unknown Ca\(^{2+}\) buffering capacity of mitochondria. By measuring [Ca\(^{2+}\)]_c and [Ca\(^{2+}\)]_m in isolated mitochondria, Wei et al.\(^{84}\) identified two modes of mitochondrial Ca\(^{2+}\) uptake, termed MCU\(_{\text{mode1}}\) and MCU\(_{\text{mode2}}\). MCU\(_{\text{mode1}}\) mediated a large and rapid change of free [Ca\(^{2+}\)]_m in response to small additions of extramitochondrial Ca\(^{2+}\). In contrast, MCU\(_{\text{mode2}}\) mediated a slow and lower affinity Ca\(^{2+}\) uptake which was capable of taking up large amounts of Ca\(^{2+}\) that led to only relatively small changes of free [Ca\(^{2+}\)]_m. The different modes can be explained by access of these two MCU modes to different Ca\(^{2+}\) buffer systems, with MCU\(_{\text{mode1}}\) allowing pronounced changes in free [Ca\(^{2+}\)]_m without major intramitochondrial Ca\(^{2+}\) buffering, potentially controlling Krebs cycle dehydrogenase activation for energy supply-and-demand matching.\(^{84}\) In contrast, MCU\(_{\text{mode2}}\) has access to a dynamic phosphate-dependent buffer system that serves as a Ca\(^{2+}\) sink in pathological situations of cytosolic Ca\(^{2+}\) overload (e.g. during ischaemia/reperfusion).\(^{84}\) In this respect, it was an interesting observation that the activation of the mPTP was uncorrelated with free [Ca\(^{2+}\)]_m and may rather be mediated by a ‘downstream by-product of [Ca\(^{2+}\)]_m, perhaps a Ca\(^{2+}\)-phosphate species’.\(^{84}\)

5. Evidence for a mitochondrial Ca\(^{2+}\) microdomain

An important question is how mitochondria can take up Ca\(^{2+}\) at rapid kinetics considering that the K_g of the MCU for Ca\(^{2+}\) is in the upper micromolar range, while the bulk cytosolic Ca\(^{2+}\) concentrations during systole are 1–2 orders of magnitude lower even during β-adrenergic stimulation. This seeming paradox would be resolved by the concept of a mitochondrial Ca\(^{2+}\) microdomain, in which mitochondria are in close vicinity to the RyRs of the SR (Figure 1 and inset), a concept that is already well accepted in non-cardiac cells with regard to the interaction between the endoplasmic reticulum (ER) and mitochondria.\(^{81}\) In fact, early electron micrographic studies revealed that in cardiac myocytes, the average distance between RyR2s and the mitochondrial surface ranges between 37 and 270 nm,\(^{103}\) and in our more recent studies analysing transmission electron micrographs, the distance between the junctional SR and mitochondria averaged
15–20 nm.\textsuperscript{68} Computational modelling predicts that the $[\text{Ca}^{2+}]$ at this distance from RyR is at least 30 \(\text{mol}\cdot\text{L}^{-1}\) during \(\text{Ca}^{2+}\) release.\textsuperscript{11} When simulating such 'microdomain \(\text{Ca}^{2+}\) transients' by exposing mitochondria to brief \(\text{Ca}^{2+}\) pulses of 10–30 \(\text{mol}\cdot\text{L}^{-1}\) in a computer model that integrates known kinetics of mitochondrial \(\text{Ca}^{2+}\) uptake and release with Krebs cycle metabolism, our experimental findings in cardiac myocytes could be reconciled.\textsuperscript{43,45} Also functional data support the existence of a mitochondrial \(\text{Ca}^{2+}\) microdomain in cardiac myocytes: in cardiac H9c2 cells, mitochondrial \(\text{Ca}^{2+}\) uptake in response to caffeine-induced rapid SR \(\text{Ca}^{2+}\) release was comparable with exposing mitochondria to an extramitochondrial $[\text{Ca}^{2+}]$ of 30 \(\text{mol}\cdot\text{L}^{-1}$.\textsuperscript{104} When adding \(\text{Ca}^{2+}\) buffers (EGTA or BAPTA) to the cytosol, the cytosolic, but not the mitochondrial \(\text{Ca}^{2+}\) transient could be suppressed after rapid caffeine-induced SR \(\text{Ca}^{2+}\) release in cardiac cells, indicating privileged \(\text{Ca}^{2+}\) communication between both organelles.\textsuperscript{103,104} In mitochondria that were isolated by standard procedures from adult hearts, the application of caffeine elicited a \(\text{Ca}^{2+}\) transient in the mitochondrial matrix, but not in the extramitochondrial solution, which could be explained by \(\text{Ca}^{2+}\) transfer from the SR still being intimately linked to the OMM.\textsuperscript{105} Two recent studies that used \(\text{Ca}^{2+}\)-sensitive fluorescent proteins genetically targeted to the OMM in non-cardiac cells or cardiac myotubes identified \(\text{Ca}^{2+}\) concentrations at the interface between the ER and mitochondria 5–10 times higher than in the bulk cytosol,\textsuperscript{106,107} further supporting the idea that mitochondria are exposed to \(\text{Ca}^{2+}\) concentrations sufficient to overcome the low \(\text{Ca}^{2+}\) affinity of the MCU.

Recently, we tested the existence and physiological implications of a mitochondrial \(\text{Ca}^{2+}\) microdomain in adult guinea-pig cardiac myocytes by comparing the efficiency of mitochondrial \(\text{Ca}^{2+}\) uptake (indexed by the ratio of $[\text{Ca}^{2+}]_{\text{m}}$ over $[\text{Ca}^{2+}]_{\text{cyt}}$) in the same cells, respectively) after either a coordinated SR \(\text{Ca}^{2+}\) release or a trans-sarcolemmal \(\text{Ca}^{2+}\) influx via the reverse-mode of the NCX with \(\text{Ca}^{2+}\)-depleted SR.\textsuperscript{100} The efficiency of mitochondrial \(\text{Ca}^{2+}\) uptake was twice as high after SR \(\text{Ca}^{2+}\) release compared with NCX-mediated \(\text{Ca}^{2+}\) influx, with a tight correlation between the rate of rise of the cytosolic \(\text{Ca}^{2+}\) transient and the efficiency of mitochondrial \(\text{Ca}^{2+}\) uptake.\textsuperscript{101} This was of bioenergetic consequence, since the ratio of NAD(P)H/FAD, a sensitive index of Krebs cycle dehydrogenase activity, was increased more efficiently after SR \(\text{Ca}^{2+}\) release than after NCX-driven \(\text{Ca}^{2+}\) influx of similar magnitude. Taken together, these data strongly support the concept of privileged \(\text{Ca}^{2+}\) communication between mitochondria and the SR over other sources for cytosolic \(\text{Ca}^{2+}\) influx.

A very recent study by the Rizzuto group\textsuperscript{85} used genetically encoded fluorescent \(\text{Ca}^{2+}\) indicators targeted to the cytosol, the OMM or the mitochondrial matrix in combination with genetic silencing or up-regulation of the MCU in neonatal cardiac myocytes and observed that (i) \(\text{Ca}^{2+}\) concentrations at the OMM clearly exceeded bulk cytosolic \(\text{Ca}^{2+}\) concentrations by several orders of magnitude, (ii) down-regulation of the MCU decreased and up-regulation of the MCU increased rapid $[\text{Ca}^{2+}]_{\text{m}}$ transients while (iii) the opposite occurred in the cytosol, where MCU down-regulation increased the amplitude of $[\text{Ca}^{2+}]_{\text{m}}$ transients and up-regulation of MCU decreased $[\text{Ca}^{2+}]_{\text{m}}$ transients. These data and some previous results\textsuperscript{43} lend further support to the concept of a mitochondrial \(\text{Ca}^{2+}\) microdomain and—in contrast to other studies\textsuperscript{75,76,106}—suggest that in cardiac myocytes, rapid mitochondrial \(\text{Ca}^{2+}\) uptake shapes cytosolic \(\text{Ca}^{2+}\) transients.

In this context, it is interesting to note that in atrial cardiac myocytes, the lack (or paucity) of T-tubules limits cytosolic \(\text{Ca}^{2+}\) transients to the subsarcolemmal space. Mackenzie et al.\textsuperscript{109} observed that the propagation of subsarcolemmal \(\text{Ca}^{2+}\) transients to the central regions of the myocytes was limited by the SR and mitochondria, since inhibition of either SERCA or mitochondrial \(\text{Ca}^{2+}\) uptake increased the propagation of cytosolic \(\text{Ca}^{2+}\) to the central regions of the cell. These data support the idea that mitochondria could serve as a spatial buffer and thus, affect the magnitude and propagation of cytosolic \(\text{Ca}^{2+}\) transients.

6. Proteins that tether mitochondria to the SR

6.1 Role of mitofusin

Electron tomography studies on adult rat cardiac myocytes identified linker proteins that directly attach mitochondria to the SR, but whose molecular identity remained unresolved.\textsuperscript{105,110} In a seminal study on mouse embryonic fibroblasts, de Brito and Scorrano\textsuperscript{67} identified mitofusin (Mfn) 2 to physically link the ER to mitochondria. Mfn5 are proteins in the OMM with a well-characterized role in mitochondrial fusion (for a recent review, see Dorn and Maack\textsuperscript{111}). Mfn1 and Mfn2 fuse mitochondria with each other by forming homo- and heterotypic complexes and thus, serve redundant roles for this process, so that deletion of neither Mfn1 nor Mfn2 alone affected mitochondrial fusion in vivo.\textsuperscript{68,112} Since Mfn2 is also located to the cardiac SR,\textsuperscript{113} it might serve as a linker between the SR and mitochondria, as revealed by de Brito and Scorrano\textsuperscript{67} for ER-mitochondrial tethering in non-cardiac cells. To test this, we analysed mice with cardiomyocyte-specific post-natal deletion of either Mfn1 or Mfn2.\textsuperscript{68} Up to an age of 6 weeks, these mice developed normal with no cardiac phenotype. However, transmission electron microscopy revealed that in Mfn2−, but not Mfn1-deficient mice, the contact length between the junctional SR and mitochondria was decreased, with a trend towards a widening of the gap between the two organelles. Likewise, RyR-containing mitochondrial associated membranes as a biochemical measure of SR-mitochondrial contact were strikingly decreased in Mfn2-KO mice.\textsuperscript{68}

These data contrast somewhat with data from the Walsh group by Papanicolaou et al.,\textsuperscript{114} who reported that cardiac-specific ablation of Mfn2 in mice did not alter the close associations between SR and mitochondria. There were, however, important methodological differences between our studies and those of Papanicolaou et al..\textsuperscript{114} First, we used a mouse model with post-natal knock-out of Mfn2,\textsuperscript{68} while in the other study,\textsuperscript{114} Mfn2 was knocked out already in the embryo, potentially facilitating mechanisms to compensate for the loss of Mfn2. Furthermore, Papanicolaou et al.,\textsuperscript{114} analysed only the distance between mitochondria and the centre of T-tubules, which was in the range of 150 nm and not different between Mfn2-KO and control animals.\textsuperscript{114} In contrast, we analysed the distance between the junctional SR and mitochondria, which is in the range of only 15 nm and a more direct parameter for SR-mitochondrial tethering.

To elucidate the functional relevance of these ultrastructural alterations, we performed experiments on isolated cardiac myocytes which were exposed to a physiological increase in workload (increased pacing frequency and \(\beta\)-adrenergic stimulation for 3 min). While in Mfn1 KO myocytes, mitochondrial \(\text{Ca}^{2+}\) uptake and the redox states of NAD(P)H and FAD were unchanged compared with WT littermates, in Mfn2-KO myocytes, mitochondrial \(\text{Ca}^{2+}\) uptake was
hampered during β-adrenergic stimulation at low stimulation frequencies. At the same time, a transient oxidation of NAD(P)H and FADH₂ occurred, indicating that reduced mitochondrial Ca²⁺ uptake was associated with decreased Krebs cycle dehydrogenase activation. In agreement with our previous observation that the Krebs cycle also controls NADPH and ROS elimination, a transient increase in ROS formation was observed in Mfn2-KO myocytes. Taken together, these results uncover critical roles for cardiomyocyte Mfn2 in tethering mitochondria to the junctional SR and creating a mitochondrial Ca²⁺ microdomain. Disruption of SR-mitochondrial cross-talk hampers energy supply-and-demand matching, and possibly provokes oxidative stress. At the same time, these data are in line with previous reports that mitochondrial Ca²⁺ uptake shapes cytosolic Ca²⁺ transients.

6.2 Other tethering proteins

Previous studies in non-cardiac cells have revealed several proteins involved in tethering the ER to mitochondria. Already in 2002, Rizzuto’s group identified voltage-dependent anion channel (VDAC), a well-characterized Ca²⁺-permeating protein in the OMM to govern Ca²⁺ transmission from the ER to mitochondria in HeLa cells. Subsequently, the same group found that VDAC is physically linked to the ER Ca²⁺ release channel, the inositol 1,4,5-triphosphate receptor (IP₃R) by glucose-regulated protein 75 (grp75). In a more recent study, Hajnoczky’s group observed that during cardiomyocyte development, the ER-mitochondrial Ca²⁺ transfer is governed by IP₃Rs in early developmental stages of cardiomyocytes transforms to a RyR2-dependent SR-mitochondrial Ca²⁺ transfer. In agreement with this concept, a recent biochemical study revealed physical interaction between RyR2 and VDAC2 with co-localization in sarco-endoplasmic reticulum microdomains between the SR and the OMM, and recent evidence suggests that the areas of the OMM which are tethered closely to the SR, OMM, and IMM is likely to ‘provide a favourable spatial arrangement for local RyR2-mitochondrial Ca²⁺ signalling’.113

7. Pathophysiological changes in heart failure

In chronic heart failure, maladaptive remodelling of EC coupling induces contractile dysfunction. The most prominent changes are (i) decreased SR Ca²⁺ load, (ii) an elevation of [Na⁺], which reduces cytosolic Ca²⁺ efflux (via the forward mode of the NCX) and increases Ca²⁺ influx (via the reverse mode; Figure 1), and (iii) t-tubular remodelling that induces a dysynchronisation of cytosolic Ca²⁺ transients. Irrespective of the absolute quantities of mitochondrial Ca²⁺ uptake during EC coupling, all these processes have a negative impact on mitochondrial Ca²⁺ uptake. First, the amplitudes of mitochondrial Ca²⁺ transients correlate with the amplitudes of cytosolic Ca²⁺ transients, which are decreasing in failing cardiac myocytes. Furthermore, we observed that reverse-mode NCX-driven Ca²⁺ influx is less efficient for mitochondrial Ca²⁺ uptake than coordinated SR Ca²⁺ release (due to its slower kinetics and less privileged localization to mitochondria; Figure 1). Similarly, t-tubular dysfunction leads to dysynchronous and thus, slower cytosolic Ca²⁺ transients, which based on our experimental results should also negatively affect mitochondrial Ca²⁺ uptake. A study on human cardiac mitoplasts revealed that the opening probability of the mCa1 (equalling MCU) and mCa2 were reduced in the mitochondria from patients with heart failure. Finally, since Ca²⁺ is exported from the mitochondria by the NCLX, the elevation of [Na⁺], observed in failing myocytes reduces steady-state [Ca²⁺]ᵢ, and leads to an oxidation of NADH and NADPH, which on the one hand impairs energy supply-and-demand matching and on the other hand leads to oxidative stress. Increased ROS production, in turn, activates CaMKII, which elevates the late Iₙa 30 and thus, [Na⁺], inducing a positive feedback-loop that sustains defects in EC coupling, energetics deficit and oxidative stress. Furthermore, mitochondrial ROS activate RyRs and thus, have a direct effect on SR Ca²⁺ release events, which may further contribute to SR Ca²⁺ leak and arrhythmias (Figure 1).60–62

8. Conclusions

Considerable progress has been made on identifying the mechanisms and molecular determinants of mitochondrial Ca²⁺ uptake and release in cardiac myocytes. In particular, the identification of the MCU, its regulatory factors MICU1 and MCU1 as well as of the NCLX, but also tethering proteins between the SR and mitochondria, such as Mfn2, RyR2, and VDAC will allow further characterization of the processes of mitochondrial Ca²⁺ uptake and release in cardiac myocytes. These studies together with the further improvement of mitochondrial Ca²⁺ indicators will hopefully resolve some of the remaining open issues such as the exact quantities and kinetics of mitochondrial Ca²⁺ uptake during EC coupling and its role for cytosolic Ca²⁺.

Conflict of interest: none declared.

Funding

The authors are supported by the Deutsche Forschungsgemeinschaft (DFG; Heisenberg Programm, SFB 894 and Klinische Forschergruppe 196).

References

Calcium release microdomains and mitochondria


