The Mitochondrial Ca^{2+} Uniporter: Structure, Function, and Pharmacology

Jyotsna Mishra, Bong Sook Jhun, Stephen Hurst, Jin O-Uchi, György Csordás, and Shey-Shing Sheu

Abstract

Mitochondrial Ca^{2+} uptake is crucial for an array of cellular functions while an imbalance can elicit cell death. In this chapter, we briefly reviewed the various modes of mitochondrial Ca^{2+} uptake and our current understanding of mitochondrial Ca^{2+} homeostasis in regards to cell physiology and pathophysiology. Further, this chapter focuses on the molecular identities, intracellular regulators as well as the pharmacology of mitochondrial Ca^{2+} uniporter complex.

Keywords

Mitochondria • Mitochondrial Ca^{2+} uniporter • Mitochondrial Ca^{2+} uptake • Pharmacology

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Jyotsna Mishra and Bong Sook Jhun contributed equally to this work.

J. Mishra • S. Hurst • S.-S. Sheu (✉)
Center for Translational Medicine, Department of Medicine, Sidney Kimmel Medical College, Thomas Jefferson University, 1020 Locust Street, Suite 543D, Philadelphia, PA 19107, USA
e-mail: shey-shing.sheu@jefferson.edu

B.S. Jhun • J. O-Uchi (✉)
Cardiovascular Research Center, Department of Medicine, Rhode Island Hospital, Warren Alpert Medical School of Brown University, Providence, RI 02903, USA
e-mail: jin.ouchi@lifespan.org; jin_o-uchi@brown.edu

G. Csordás (✉)
MitoCare Center for Mitochondrial Imaging Research and Diagnostics, Department of Pathology, Anatomy and Cell Biology, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA 19107, USA
e-mail: Gyorgy.Csordas@jefferson.edu

Handbook of Experimental Pharmacology, DOI 10.1007/164_2017_1

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Abbreviations

$[Ca^{2+}]_c$	Cytosolic Ca^{2+} concentrations
$[Ca^{2+}]_m$	Mitochondrial Ca^{2+} concentrations
$[Ca^{2+}]_o$	Extramitochondrial free Ca^{2+} concentrations
ATP	Adenosine triphosphate
CaMK	Ca^{2+}/calmodulin kinase
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
CoQ10	Coenzyme Q 10
CREB	Cyclic adenosine monophosphate response element–binding protein
EMRE	Essential MCU regulator
ER/SR	Endoplasmic reticulum/Sarcoplasmic reticulum
ICDH	Isocitrate dehydrogenase
IMM	Inner mitochondrial membrane
IMS	Intermembrane space
IP_3R	Inositol 1,4,5-trisphosphate receptor
LETM1	Leucine zipper-EF-hand containing transmembrane protein 1
MAPK	Mitogen-activated protein kinase
MCU	Mitochondrial Ca^{2+} uniporter pore
MCUR1	Mitochondrial Ca^{2+} uniporter regulator 1
MICU1	Mitochondrial Ca^{2+} uptake 1
MICU2	Mitochondrial Ca^{2+} uptake 2
MICU3	Mitochondrial Ca^{2+} uptake 3
mPTP	Mitochondrial permeability transition pore
mRyR1	Mitochondrial ryanodine receptor 1
mtCU	Mitochondrial Ca^{2+} uniporter
NMR	Nuclear magnetic resonance
Npas4	Neuronal PAS Domain Protein 4

OGDH	α-Ketoglutarate/oxoglutarate dehydrogenase
OMM	Outer mitochondrial membrane
PDH	Pyruvate dehydrogenase
Pyk2	Proline-rich tyrosine kinase 2
RaM	Rapid mode of uptake
ROS	Reactive Oxygen Species
Ru360	Ruthenium 360
RyR	Ryanodine receptor
TASK-3	TWIK-related Acid-sensitive K^+ channel
TRPC3	Transient receptor potential channel 3
TRPV	Transient Receptor Potential Vanilloid
VDAC	Voltage dependent anion channel
α_1-AR	α1-Adrenoceptor

1 Introduction

Mitochondria play an important role in Ca^{2+} homeostasis, which is crucial for balancing cell survival and death (Giacomello et al. 2007; Duchen et al. 2008). During the 1950s it was observed that isolated mitochondria could accumulate Ca^{2+} (Carafoli 2010). Subsequently, an energy-driven accumulation of Ca^{2+} by isolated mitochondria was demonstrated (Vasington and Murphy 1962; Deluca and Engstrom 1961). It was initially thought that mitochondrial Ca^{2+} transport consists of an active uptake and passive release process (Chance 1965), but multiple groups [reviewed by Gunter et al. (1994)] showed that Ca^{2+} uptake across the inner mitochondrial membrane (IMM) is energetically favorable, while efflux requires electrogenic ion-exchange (antiport). This raised the possibility that mitochondria may play a significant role in the regulation or buffering of cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_c$) (Nicholls 1978). Though, mitochondria were one of the first organelle to be associated with intracellular Ca^{2+} handling, the relative low affinity of their Ca^{2+} transport systems led to the conclusion that they were physiologically irrelevant. It was demonstrated that in suspensions of respiring isolated rat liver mitochondria alone, the steady state extramitochondrial free Ca^{2+} concentrations ($[Ca^{2+}]_o$) of incubating solutions were about 0.5 μM (Becker et al. 1980). Addition of microsomes, which contain endoplasmic reticulum (ER) that has Ca^{2+} transport systems with a higher affinity for Ca^{2+} than that of mitochondria, was able to reduce $[Ca^{2+}]_o$ to 0.2 μM. Similar results were obtained in digitonin-permeabilized hepatocytes and thus brought forth the idea that the "set point" of $[Ca^{2+}]_c$ is established by the ER Ca^{2+} transport mechanisms and not the mitochondria (at ~0.2 μM) (Becker et al. 1980). However, interest revived in mitochondrial Ca^{2+} homeostasis in the 1990s when the development of Ca^{2+} sensors that can selectively measure the changes in the mitochondrial matrix Ca^{2+} concentrations ($[Ca^{2+}]_m$) allowed to demonstrate propagation of physiological Ca^{2+} signals from cytosol into the mitochondrial matrix. High Ca^{2+} microdomains at the ER/sarcoplasmic reticulum (SR) and mitochondria interface addressed the discrepancy between the relatively small (approximately 1 μM or less) global $[Ca^{2+}]_c$ peak levels and the much higher

in vitro activation range ($K_d \cong 50$ μM) for the mitochondrial Ca^{2+} uniporter (mtCU) in most tissues. The ER/SR, which possesses the Ca^{2+}-release channels, inositol 1,4,5-trisphosphate receptor (IP_3R), and/or ryanodine receptor (RyR), could release Ca^{2+} at the mitochondria/ER/SR junctions with concentrations sufficient to meet the threshold of the mtCU (Rizzuto and Pozzan 2006; O-Uchi et al. 2012). These groundbreaking studies repositioned mitochondria as key players in the dynamic regulation of cellular Ca^{2+} signaling under physiological conditions.

Ca^{2+} uptake into mitochondria was mostly considered to result from a single transport mechanism mediated by a Ca^{2+}-selective channel of the IMM, the mtCU (Gunter and Pfeiffer 1990). The electrophysiological characteristic of mtCU as a highly selective Ca^{2+} activated Ca^{2+} channel (I_{MiCa}) was confirmed by measuring total or single-channel ionic current from the IMM of mitoplasts (Kirichok et al. 2004). The discovery of the molecular identity of the mtCU protein complexes was tightly connected to the establishment of MitoCarta, a comprehensive mitochondrial protein compendium in 2008 (Pagliarini et al. 2008). Based on the establishment of this compendium, the Ca^{2+} sensing EF-hand regulator mitochondrial Ca^{2+} uptake 1 (MICU1) was identified first in 2010 as a regulator of the channel (Perocchi et al. 2010). With one or no predicted transmembrane domain, MICU1 has never been considered to form the mtCU pore. To that end, in 2011, a ~40 kDa protein with two transmembrane domains was discovered as the molecular identity of the mtCU pore termed MCU by the groups of Mootha and Rizzuto (De Stefani et al. 2011; Baughman et al. 2011). Following the identification of the MCU, other regulatory subunits were identified in the last 5 years. These findings open up exciting opportunities for using genetic approaches to elucidate molecular mechanisms that regulate mitochondrial Ca^{2+} uptake in a variety of cell types/tissues. Since the mechanisms for regulating mitochondrial Ca^{2+} concentrations ($[Ca^{2+}]_m$) are critical for fundamental cellular processes, the importance of understanding Ca^{2+} uptake mechanisms in physiology (Tarasov et al. 2012; Alam et al. 2012; Xu and Chisholm 2014) and pathophysiology (Mallilankaraman et al. 2012a; Huang et al. 2013; Csordas et al. 2013; Hall et al. 2014) has become increasingly relevant.

In this chapter, we review the current model of the mitochondrial Ca^{2+} influx mechanism, with special focus on the molecular identity of the mtCU complex.

Furthermore, the physiological, pathophysiological, and pharmacological implications of mitochondrial Ca^{2+} uptake and future directions of study are discussed.

2 Molecular Identities of Mitochondrial Ca^{2+} Channels/Transporters

2.1 Overview

Following the discovery of the pore, MCU, further regulatory subunits were identified, suggesting that the mtCU exists as a multi-protein complex capable of multiple states of MCU activity (De Stefani et al. 2011). Proteins in the mtCU

complex include transmembrane subunits [MCU, MCUb, and the essential MCU regulator (EMRE)], and membrane-associated regulatory subunits in the intermembrane space (IMS) (MICU1-3) (Fig. 1). Mitochondrial Ca^{2+} uniporter regulator 1 (MCUR1), another two transmembrane domain coiled-coil domain containing protein of the IMM was also proposed to interact with the MCU protein and to modulate the channel function (Mallilankaraman et al. 2012b); however, it was not present in the ~480 kDa uniporter holocomplex coined as the "uniplex" (Sancak et al. 2013). In addition to mtCU complex, we also briefly describe other mitochondrial Ca^{2+} channels/transporters that have been reported, which includes mitochondrial ryanodine receptor 1 (mRyR1), rapid mode of uptake (RaM), mCa1 and 2, Coenzyme Q 10 (CoQ10), the transient receptor potential channel 3 (TRPC3), and the Leucine zipper-EF-hand containing transmembrane protein 1 (LETM1).

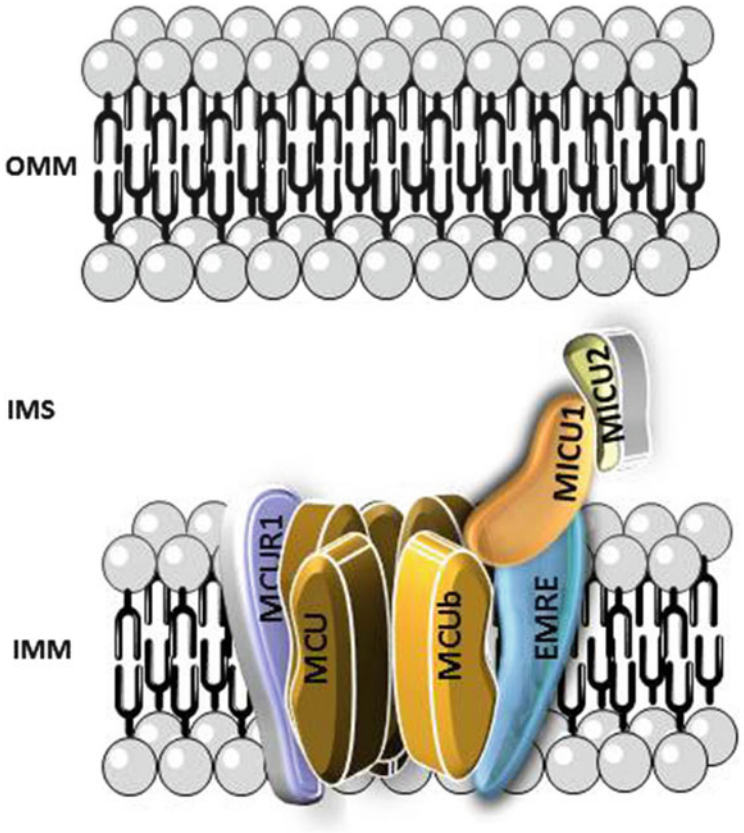

Fig. 1 The molecular structure of the mtCU complex. Composed of MCU and MCUb (the channel forming subunits) together with essential mtCU regulators, EMRE, MCUR1 and intermembrane space proteins, MICU1 and MICU2

2.2 mtCU Complex

2.2.1 MCU

The MCU gene (previously known as CCDC109A) is highly conserved across eukaryotes except yeast (De Stefani et al. 2011; Baughman et al. 2011). The MCU is a 40 kD protein that contains a proteolytically cleaved mitochondrial import sequence, two coiled-coil domains, two transmembrane domains, and a short motif of amino acids between the two transmembrane domains critical for Ca^{2+} transport (De Stefani et al. 2011; Baughman et al. 2011). MCU has been suggested to form the pore as a homo-oligomer and a recent study using nuclear magnetic resonance (NMR) demonstrated a pentameric stoichiometry (Oxenoid et al. 2016). Although there was originally some debate about the MCU topology, it is clear now that both the N- and C-termini face the mitochondrial matrix with a short motif of amino acids being exposed to the IMS (Martell et al. 2012). Overexpression of MCU increases the rate of mitochondrial Ca^{2+} influx in both intact and permeabilized cells, causing a significant decrease in $[Ca^{2+}]_c$ transients in intact cells (De Stefani et al. 2011). Further, the mutation of two negatively charged residues inside the highly conserved DIME motif (QxGxLAxLTWWxYSWDIMEPVTYF), in the IMS (D261Q/E264Q in human MCU) completely abolishes the MCU activity (De Stefani et al. 2011; Baughman et al. 2011). On the other hand, the partial knockdown of MCU greatly inhibits the rate and amplitude of mitochondrial Ca^{2+} entry (De Stefani et al. 2011; Baughman et al. 2011) whereas the knockout essentially eliminates rapid uptake of Ca^{2+} pulses (Sancak et al. 2013; Pan et al. 2013) and the expression of the wild-type MCU in MCU knockdown cells fully rescues Ca^{2+} uptake profile (Baughman et al. 2011). Thus, MCU is responsible for Ca^{2+} transport into the mitochondria. As of now, the essential role of MCU for mitochondrial Ca^{2+} uptake was validated in many cell types/tissues including the liver (Baughman et al. 2011), heart (Joiner et al. 2012), cardiomyocytes (Drago et al. 2012; O-Uchi et al. 2014), skeletal muscles (Pan et al. 2013), pancreatic β cells (Tarasov et al. 2012), neurons (Qiu et al. 2013), and mammary gland epithelial cells (Hall et al. 2014).

2.2.2 MCUb

MCUb, originally reported as CCDC109B, is a 33-kDa protein that shares 50% similarity to MCU with the key amino acid substitutions (R251W, E256V) in the DIME motif (Raffaello et al. 2013). Co-introduction of MCU and MCUb in a lipid bilayer dramatically decreases the open probability compared to only MCU incorporation. In addition, MCUb overexpression in intact cells decreases mitochondrial Ca^{2+} uptake in response to $[Ca^{2+}]_c$ increases, suggesting that MCUb interacts with MCU and acts as an endogenous dominant-negative subunit of the mtCU pore (Raffaello et al. 2013). Interestingly, the ratio of the amount of MCU and MCUb mRNA varies in different tissues (Sancak et al. 2013; Raffaello et al. 2013; Fieni et al. 2012). This raises the possibility that the ratio of MCU and MCUb expression may be one of the mechanisms that differentially regulate mitochondrial Ca^{2+} uptake in different tissues.

2.2.3 MICU1-3

MICU1 (previously known as CBARA1/EFHA3) is a 54-kDa protein with two highly conserved EF-hand Ca^{2+}-binding domains (Perocchi et al. 2010). The sub-mitochondrial localization of MICU1 has been a matter of debate (Perocchi et al. 2010; Mallilankaraman et al. 2012a; Hoffman et al. 2013) but recent proteomic mapping studies (Hung et al. 2014; Lam et al. 2015) as well as interactome analysis of the intermembrane space oxidoreductase MIA40 (Petrungaro et al. 2015) indicate that the MICU1 is a soluble (or membrane associated) protein in the IMS (Csordas et al. 2013; Patron et al. 2014; Wang et al. 2014), but not in the matrix. MICU1 is proposed to be pivotal in both the gatekeeping and cooperative activation of mtCU; keeping the channel closed at resting conditions, but promoting cooperative activation of the channel at high Ca^{2+} (Csordas et al. 2013; de la Fuente et al. 2014). Alternatively, MICU1 was also proposed to only convey either of these functions (gatekeeper (Mallilankaraman et al. 2012a; Hoffman et al. 2013), cooperative activator) (Patron et al. 2014).

Additionally, MICU isoforms, MICU2 (known as EFHA1) and MICU3 (known as EFHA2) are also identified (Plovanich et al. 2013). Both MICU2 and MICU3 possess the conserved EF-hand domains, but share only 25% sequence identity with MICU1 (Plovanich et al. 2013). Relative expression levels of these MICU isoforms vary across the different tissue types. MICU1 and MICU2 are ubiquitously expressed in mammalian tissues, whereas MICU3 is expressed only in the nervous system and skeletal muscle (Plovanich et al. 2013). Though the role of MICU1 and MICU2 have been extensively studied by several groups, but up to date there is no report attempted to characterize the MICU3 function. MICU2 forms a heterodimer with MICU1, thus indirectly associating with the MCU (Patron et al. 2014; Plovanich et al. 2013). Moreover, the stability of MICU2 is dependent on the level of MICU1 expression (Patron et al. 2014; Plovanich et al. 2013). Importantly, MICU2 inhibits the function of the MCU at lower $[Ca^{2+}]_c$ levels both in planar lipid bilayers and in intact cells (Patron et al. 2014; Matesanz-Isabel et al. 2016). These data lead to the suggestion that MICU2 would be the gatekeeper of MCU instead of MICU1, which would form a regulatory dimer with MICU2 to modulate MCU channel activity in opposite manner. On the other hand, a recent study by the Mootha group showed that upon disabling the Ca^{2+} sensing by their EF hands, MICU1 and MICU2 both would keep the channel closed and MICU1 would do this even if MICU2 was ablated (MICU2 KO) (Kamer and Mootha 2014). This would suggest that MICU1 alone can act as a gatekeeper but the gatekeeping activity would be lifted by lower $[Ca^{2+}]$ than that of MICU2 (Matesanz-Isabel et al. 2016). At low $[Ca^{2+}]_c$, the inhibitory effect of MICU2 is in dominance to safeguard minimal Ca^{2+} accumulation in the presence of a very large electromotive force for cation accumulation. At high $[Ca^{2+}]_c$, however, Ca^{2+}-dependent MICU2 inhibition and MICU1 activation warrant the mitochondria to respond rapidly for bringing adequate amount of Ca^{2+} into matrix during $[Ca^{2+}]_c$ oscillations so that Ca^{2+}-sensitive steps in ATP production can be stimulated efficiently. A very recent work by the Rizzuto/Raffaello group describes a splice variant of MICU1, termed MICU1.1 containing an insertion of 4 amino acids (EFWQ) at position

181 of MICU1, that is highly expressed in the skeletal muscle with increased Ca^{2+} binding affinity (Vecellio Reane et al. 2016). This splice variant seems to convey higher sensitivity (lower threshold) for the activation of mtCU further suggesting that MICU1 is instrumental in the gatekeeping of mtCU.

2.2.4 EMRE

EMRE (known as C22ORF32) is a 10-kDa protein that contains a single transmembrane domain and a highly conserved aspartate-rich C-terminal region (Sancak et al. 2013). While MCU and MICUs are well preserved across phylum, EMRE homologs are not present in plants, fungi, or protozoa, indicating that EMRE likely arose in the metazoan lineage (Sancak et al. 2013). However, within mammals, EMRE is ubiquitously expressed across tissues (Sancak et al. 2013). Importantly, it has been shown that knockdown or knockout of EMRE completely abolishes mitochondrial Ca^{2+} uptake, indicating that this protein is essential for the functional mtCU channel. EMRE interacts with MCU at the IMM and MICU1 at the IMS, acting as a retainer of MICU1/2 in the mtCU complex (Sancak et al. 2013; Kovacs-Bogdan et al. 2014; Tsai et al. 2016; Yamamoto et al. 2016; Vais et al. 2016). A majority of evidence suggest that the N-terminus of EMRE faces the matrix with the C-terminus facing the IMS (Tsai et al. 2016; Yamamoto et al. 2016; Tomar et al. 2016).

In addition to the $[Ca^{2+}]_c$ sensing via MICU, MCU may also be regulated by Ca^{2+} and Mg^{2+} from the matrix side. Recent work from the Foskett group has presented electrophysiological (mitoplast patch clamp) evidence for a biphasic (bell-shaped) Ca^{2+} regulation of mtCU from the matrix side with a matrix $[Ca^{2+}]$ activation window of ~0.01–2 μM. The acidic tail of EMRE was shown to be critical for this $[Ca^{2+}]$ regulation from the matrix side and, contrasting other works, was suggested that EMRE would rather have an N_{out}-C_{in} topology and its acidic tail would operate as the luminal Ca^{2+} sensor. Since MICU1/2 were also required for the matrix-side $[Ca^{2+}]$ regulation and considering the overwhelming evidence for EMRE's N_{in}-C_{out} topology, one could entertain an alternative mechanism for EMRE's contribution. EMRE may relay a signal from a distinct matrix Ca^{2+} sensor to the gatekeepers MICU1/2 via the interaction of its C-terminal acidic tail with a lysine-rich basic stretch of MICU1 (Tsai et al. 2016). As to the matrix Ca^{2+} sensor, very recently a comprehensive molecular structure (crystallography) study has identified a Ca^{2+}/Mg^{2+} binding acidic patch on the N-terminal matrix domain of MCU that conveys Mg^{2+} dependent inactivation of the channel (Lee et al. 2016). Further studies will be needed to clarify EMRE's role if any in this latter regulation.

2.2.5 MCUR1

MCUR1 (known as CCDC90A) is a 40-kDa protein that consists of two transmembrane domains and one coiled-coil region. The N- and C-termini of MICUR1 are proposed to face the IMS, thus the bulk of this protein exposed to the matrix (Mallilankaraman et al. 2012b). Knockdown of MCUR1 not only inhibits agonist-induced mitochondrial Ca^{2+} uptake, but also decreases basal $[Ca^{2+}]_m$. Overexpression of MCUR1 results in an increase of mitochondrial Ca^{2+} uptake,

but only when MCU exists, indicating that MCUR1 is required for Ca^{2+} uptake through the mtCU complex. MCUR1 interacts with MCU, but not with MICU1, suggesting that different compositions of the mtCU complex may exist. Shoubridge and colleagues raised a question about the direct involvement of MCUR1 in the regulation of the MCU complex (Paupe et al. 2015). They demonstrated that MCUR1 knockdown causes a drop of mitochondrial membrane potential ($\Delta\Psi_m$), proposed that the effect of MCUR1 on MCU activity may be indirect through changing the driving force of Ca^{2+} entry (Paupe et al. 2015). However, it was demonstrated that MCUR1 binds to the MCU-pore and EMRE through their coiled-coil domains which stabilizes the mtCU complex and loss of MCUR1 reduces the bioenergetics and promotes autophagy (Tomar et al. 2016). However, a recent study has shown that Drosophila cells lacking the MCUR1 homologue still exhibited typical mtCU Ca^{2+} uptake (Chaudhuri et al. 2016).

2.3 Other Channels

2.3.1 Transport Across the Outer Mitochondria Membrane

In order for Ca^{2+} to interact with the mtCU it must first travel across the outer mitochondrial membrane (OMM). Initially the OMM was considered to be freely permeable to Ca^{2+} mostly by way of the highly abundant voltage dependent anion channel (VDAC). Later, a pair of studies demonstrated that increasing the permeability of the OMM via overexpression of VDAC (Rapizzi et al. 2002) or via treatment with truncated Bid (tcBid) (Csordas et al. 2002) increased the rate of Ca^{2+} influx into the mitochondrial matrix from IP_3R-linked high [Ca^{2+}] microdomains. Moreover, it has been shown that physiological [Ca^{2+}] changes can enhance the cation (e.g., K^+) conductance of VDAC reconstituted in bilayer and also enhance the permeability of the OMM to H^+ and ATP in permeabilized cells (Bathori et al. 2006). Nevertheless, the cation permeability of VDAC reconstituted in a lipid bilayer has been reported higher in the closed state (Tan and Colombini 2007). Thus, VDAC expression levels as well as gating state can modulate mitochondrial Ca^{2+} entry.

2.3.2 mRyR1

Localized in the IMM, mRyR1 is an alternative mechanism for mitochondrial Ca^{2+} uptake in cardiac and neuronal cells (Jakob et al. 2014; Beutner et al. 2001, 2005). RyRs are the largest known ion channels of about >2 MDa. Three different subtypes of RyR isoforms (RyR1, RyR2, and RyR3) have been described and cloned, with different pharmacological properties and tissue-specific expression. RyR1, the primary isoform in the skeletal muscle, is considered to be the major Ca^{2+} release channel in SR (Marks et al. 1989); RyR2 is most abundant in cardiac muscle cells (Nakai et al. 1990) (and, in a lesser amount, the brain); RyR3 is widely expressed in the ER of different vertebrate tissues (Giannini et al. 1995) and may be coexpressed with RyR1 and RyR2. In cardiac muscle cells RyR2 is abundantly localized in the SR (Lanner et al. 2010), but RyR1 is also detectable both at the mRNA and protein levels (Munch et al. 2000; Jeyakumar et al. 2002).

Using immuno-gold particle and electron microscopy, we reported that a low level of RyR1 is expressed at the IMM in cardiomyocytes, and with higher Ca^{2+} conductance and higher K_m for Ca^{2+} binding as compared to mtCU, mitochondrial RyR (mRyR) channels serve as a fast and high affinity Ca^{2+} uptake pathway (Beutner et al. 2001, 2005). Owing to the remarkable biochemical, pharmacological, and functional similarity of RyR in cardiac mitochondria to those of RyR1 in skeletal muscle SR, we designated it as mRyR1 (Beutner et al. 2005). mRyR1 showed a bell-shaped Ca^{2+} dependence of [3H]ryanodine binding with maximal binding at approximately pCa of 4.4 and complete block at pCa2 suggestive of RyR1. Moreover, unlike the cardiac SR-RyR2, caffeine showed hardly any effect on ryanodine binding in mitochondria and binding was inhibited by 50% in the presence of 0.33 mmol L^{-1} Mg^{2+} (Zimanyi and Pessah 1991). In permeabilized cardiomyocytes, ruthenium red at a concentration of 1–5 μmol L^{-1} blocked mitochondrial Ca^{2+} uptake with no significant effect on SR Ca^{2+} release (Sharma et al. 2000). Single-channel characterization of the mRyR1 revealed a novel 225-pS cation-selective channel in heart mitoplasts, with 4 distinct channel conductance (100, 225, 700, and 1,000 pS in symmetrical 150 mmol L^{-1} CsCl), which was blocked by high concentrations of ruthenium red and ryanodine, known inhibitors of ryanodine receptors (Ryu et al. 2011). Ryanodine showed a concentration-dependent modulation of this channel, with low concentrations (10 μmol L^{-1}) stabilizing a subconductance state while high concentrations ($\geq$100 μmol L^{-1}) blocked the channel activity (Ryu et al. 2011).

Although both the mRyR1 and the MCU are inhibited by low concentrations of ruthenium red (1–5 μM) and Mg^{2+}, the unique single-channel characteristics of mRyR1 clearly differentiate it from previously identified mitochondrial ion channels. Further clarifications will be needed to distinct the roles of mRyR1 and mtCU in the physiological Ca^{2+} signaling activities of the cardiac muscle mitochondria. Interestingly, a recent paper shows that stimulation of IP_3R in adult cardiac myocytes with endothelin-1 causes Ca^{2+} release from the SR, which is uniquely tunneled to mitochondria via mRyR leading to stimulation of mitochondrial ATP production (Seidlmayer et al. 2016).

2.3.3 RaM

RaM, first studied in isolated liver mitochondria, is a kinetically distinct mode of mitochondrial Ca^{2+} uptake, capable of sequestering significant amounts of Ca^{2+} hundreds of times faster than the mtCU. RaM is activated only transiently, facilitates mitochondria to rapidly sequester Ca^{2+} at the beginning of each cytosolic Ca^{2+} pulse, and rapidly recovers between pulses, which allows mitochondria to respond to repetitive Ca^{2+} transients (Sparagna et al. 1995). Similar to mtCU and mRyR1, RaM was inhibited by ruthenium red, but required over an order of magnitude more than that required for the inhibition of mtCU (0.1 mmol L^{-1}). Likewise, RaM is also activated by polyamines, such as spermine, at a concentration of 0.1 mmol L^{-1} and displayed 3 times more of an increase in activity than mtCU (Gunter and Gunter 2001). In addition, a rapid mode of Ca^{2+} uptake was also proposed in isolated heart mitochondria but with some different transport features

from those of liver (Buntinas et al. 2001). The reset time was longer (>60 s) and with less sensitivity towards the inhibition by ruthenium red. Moreover, ATP and GTP activated RaM in liver but not in heart where RaM is activated by ADP and inhibited by AMP. Notably, RaM has always been considered to be potentially an "operating mode" of the uniporter instead of a distinct channel/transporter entity; however, there have been no studies to reconcile RaM with I_{MiCa} or with the thus far identified molecular components of the mtCU complex.

2.3.4 mCa 1 and 2

mCa1 and mCa2 are both voltage gated mitochondrial Ca^{2+} selective channels similar to mtCU with a maximal conductance of 10.9 and 6.56 pS, respectively, at 105 mmol L^{-1} [Ca^{2+}], and half saturating concentration (K_m) of 15.1 and 19.6 mmol L^{-1} [Ca^{2+}], respectively. They have unique single-channel characteristics and sensitivity to Ru360. mCa1 channels display higher single-channel amplitude, smaller opening time, a lower open probability ($P_O = 0.053$), and multiple sub-conductance states. While, mCa2 channels have a smaller single-channel amplitude with a lower conductance, longer openings, a higher open probability, and no sub-conductance states. Like MCU and RaM, both mCa1 and mCa2 were activated by spermine. However, mCa2 was only partially inhibited by μmol L^{-1} concentrations of Ru360 (Michels et al. 2009). Like RaM, mCa1/2 have not been studied further in the molecular era of mtCU to explore if it was indeed a distinct channel entity or rather the result of a particular (stoichiometric) permutation and/or post-translational modification of the mtCU complex constituents.

2.3.5 CoQ

CoQ10 is an essential component of the mitochondrial electron-transport chain (ETC) with the primary role as an electron and proton transporter. It was also reported that CoQ10 is a regulator of mitochondrial Ca^{2+} and redox homeostasis. Under physiological conditions, hydroxyl CoQs can bind and efficiently transport Ca^{2+}. Hydroxyl CoQs have a very high affinity for Ca^{2+} and therefore, can function at $[Ca^{2+}]_c$ lower than 0.5 μM and potentially even at resting $[Ca^{2+}]_c$ levels (Bogeski et al. 2011). This relatively slower Ca^{2+} transfer might be a component of the thus far unidentified source of small tonic Ca^{2+} accumulation observed in MCU knock-out cardiac mitochondria (Kwong et al. 2015; Luongo et al. 2015).

2.3.6 LETM1 and TRPC3

LETM1, initially identified as a K^+/H^+ exchanger, was recently reported as a Ca^{2+}/H^+ antiporter. Using an siRNA genome-wide screening in drosophila, it was reported to be localized at the IMM. It transports Ca^{2+} bidirectionally across the IMM in a pH gradient-dependent manner and is inhibited by ruthenium red (Jiang et al. 2009). However, a recent study with LETM1 protein reconstituted in liposomes demonstrated LETM1 as an electroneutral $1Ca^{2+}/2H^+$ antiporter, insensitive to ruthenium red (Tsai et al. 2014).

Lastly, TRPC3 was demonstrated as an alternative mitochondrial Ca^{2+} uptake pathway. It is permeable to Ca^{2+}, Na^+, and K^+ and can contribute to mitochondrial

Ca^{2+} uptake during conditions with a relatively high extramitochondrial [Ca^{2+}] (Feng et al. 2013).

3 Transcriptional/Post-transcriptional and Post-translational Regulation of the mtCU Complex

As described above (see Sect. 2), the mtCU is a multisubunit complex with many regulators. However, the expression patterns of each component are variable in a tissue-specific manner (Plovanich et al. 2013; Murgia and Rizzuto 2015) for adapting to the appropriate Ca^{2+} sensitivity by intracellular signals in each tissue. Therefore, it is of interest to elucidate how the mtCU complex is differentially regulated at the level of gene expression, which is linked to its modulation of mitochondrial Ca^{2+} uptake. Accordingly, it has been reported that transcriptional and post-transcriptional mechanisms can regulate MCU expression and activity to specific functional demands (Plovanich et al. 2013; Murgia and Rizzuto 2015; Marchi et al. 2013). For example, in neurons, synaptic activity suppresses MCU transcription through a nuclear Ca^{2+} signals, Ca^{2+}/calmodulin kinase (CaMK), and the transcription factor Npas4 dependent mechanism, preventing excitotoxic death (Qiu et al. 2013). In addition, the Ca^{2+}-regulated transcription factor cyclic adenosine monophosphate response element–binding protein (CREB) directly binds to the MCU promoter and stimulates MCU expression, regulating mitochondrial metabolism (Shanmughapriya et al. 2015). MCUb expression was also reported to be increased though independent of CREB activation (Shanmughapriya et al. 2015). It has been shown that MCU is also a target of microRNA-25 (miR-25), which can efficiently decrease MCU gene expression and activity (Marchi et al. 2013). Furthermore, analyses of post-translational modifications of the MCU components are ongoing. In 2012, Joiner et al. for the first time reported two Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) phosphorylation candidate motifs at the N-terminus of MCU. CaMKII resides endogenously in the mitochondrial matrix and is highly activated during pathophysiological conditions like ischemia reperfusion and myocardial infarction; promotes myocardial death via CaMKII-mediated increases in MCU current, by phosphorylation of MCU at serine 57 and 92. However, mitochondrial CaMKII inhibition reduced MCU current and was protective against ischemia/reperfusion injury, myocardial infarction, and neurohumoral injury (Joiner et al. 2012, 2014; Fieni et al. 2014). Recently, Lee et al. showed that MCU-S92A mutant expression failed to rescue the Ca^{2+} channel activity in an MCU knockdown cell line. In addition, they also presented the crystal structure of the N-terminal region of MCU including (S92) a potential CaMKII phosphorylation site and concluded them to be indispensable for modulation of channel activity (Lee et al. 2015). Additionally, our group demonstrated that α1-adrenoceptor (α1-AR) signaling activates Ca^{2+} and ROS dependent proline-rich tyrosine kinase 2 (Pyk2); translocates Pyk2 into the mitochondrial matrix. Activated Pyk2 interacts with MCU and directly phosphorylates MCU tyrosine residue(s) and enhances mitochondrial Ca^{2+} uptake by promoting MCU channel

oligomerization and formation of tetrameric channels (O-Uchi et al. 2014). However, persistent α1-AR stimulation increases ROS production, activates the mitochondrial permeability transition pore (mPTP) opening, and eventually leads to cell death via Pyk2 activation in cardiomyocytes (O-Uchi et al. 2014).

4 Physiological Roles of Mitochondrial Ca^{2+} Uptake

Mitochondrial Ca^{2+} has been implicated as an important regulator of fundamental cellular processes, which range from the regulation of cellular metabolism, buffering cytosolic Ca^{2+}, modulating cellular redox environments, to other cell-type specific functions. As described above, we have witnessed a rapid advance in our understanding of the role of mitochondrial Ca^{2+} uptake mechanisms in physiology and pathophysiology since the recent molecular discovery of the mtCU pore (i.e., MCU) and its regulators. Therefore, in the next sections, we summarize the role of mitochondrial Ca^{2+} uptake mechanisms highlighting the functions of the mtCU complex during physiological (Sect. 4) and pathological (Sect. 5) conditions.

4.1 Mitochondrial Ca^{2+} and Energy Metabolism

Mitochondrial Ca^{2+} uptake serves as one of the major factors for regulating cellular bioenergetics (Denton and McCormack 1980; Hajnoczky et al. 1995). Denton and McCormick in the 1980s demonstrated that mitochondrial Ca^{2+} plays an important role in regulating three Ca^{2+} dependent dehydrogenases: pyruvate dehydrogenase (PDH), α-ketoglutarate (also called oxoglutarate) dehydrogenase (OGDH), and NAD-isocitrate dehydrogenase (ICDH) (Denton 2009; McCormack et al. 1990) that are the rate-limiting enzymes in substrate supply for ATP synthesis (Jouaville et al. 1999). Of the three dehydrogenases, ICDH and OGDH are activated through the binding of Ca^{2+} (Rutter and Denton 1988) whereas, PDH activation depends on Ca^{2+}-dependent phosphatase mediated dephosphorylation step (Denton et al. 1972). Increase in mitochondrial Ca^{2+} uptake can activate oxidative metabolism via activated matrix dehydrogenases, resulting in an increased supply of reducing equivalents to drive respiratory chain activity and ATP synthesis (McCormack et al. 1990). Mitochondrial matrix Ca^{2+} also regulates bioenergetics by S100A1 mediated direct Ca^{2+}-dependent activation of F_0-F_1ATP synthase activity (Boerries et al. 2007; Glancy and Balaban 2012).

Surprisingly, mouse embryonic fibroblasts or isolated mitochondria from MCU-knockout mice have apparently well-maintained basal mitochondrial metabolic function and energetics, albeit with decreased Ca^{2+} uptake and lower resting Ca^{2+} levels (Perocchi et al. 2010; De Stefani et al. 2011; Baughman et al. 2011; Mallilankaraman et al. 2012b). Even more surprisingly, this lack of energetic phenotype extends to the beating heart in vivo under physiological conditions (approximately 500 beats/min), either in germline or inducible cardiac-specific

MCU knockout mice (Pan et al. 2013; Kwong et al. 2015; Luongo et al. 2015; Murphy et al. 2014). Likewise, though global MCU knockout displayed no evidence of Ca^{2+} uptake in mitochondria yet, basal ATP levels were not evidently altered, indicating that lack of MCU does not have marked impact on basal mitochondrial metabolism (Holmstrom et al. 2015). However, skeletal muscle showed a minor defect in muscle strength after endurance training (Pan et al. 2013). The mild phenotype of MCU knockout mice could be due to some kinds of adaptation in these animals (Murphy et al. 2014). Similarly, in a cardiac-specific MCU knockout mouse, there is no energetic phenotype in vivo under normal physiological conditions. However, these mice displayed a decreased β-adrenergic receptor-mediated fight or flight response for increased workload under stress and a decreased ischemia-reperfusion injury (Kwong et al. 2015; Luongo et al. 2015). Similar results have been obtained via cardiac-specific overexpression of a dominant-negative mutant MCU (Wu et al. 2015). These surprising findings have set a stage for seeking other compensatory or unknown mechanisms for the MCU-independent regulation of bioenergetics in beating heart (Harrington and Murphy 2015).

Knockdown of MCUR1 reduces mitochondrial Ca^{2+} uptake resulting in disruption of oxidative phosphorylation which activates AMP kinase-dependent pro-survival autophagy (Mallilankaraman et al. 2012b). However, in pancreatic β-cells, knockdown of MCU and MICU1 markedly reduced the mitochondrial Ca^{2+} uptake and showed that MCU- and MICU1-mediated Ca^{2+} uptake is critical for continual ATP synthesis, glucose metabolism, and insulin secretion (Tarasov et al. 2012; Alam et al. 2012). MCU silencing down-regulates the expression of respiratory chain complexes, mitochondrial metabolic activity, and oxygen consumption (Quan et al. 2015). In addition to MCU, absence of LETM1 decreased basal mitochondrial oxygen consumption, discernible inactivation of complex IV activity, and a drop in ATP production (Doonan et al. 2014). We recently reported that RyR1-overexpressing cardiac cells had higher mitochondrial ATP under basal conditions with augmented $[Ca^{2+}]_c$-dependent ATP production (O-Uchi et al. 2013), supporting our previous finding of a low respiratory control index in RyR1 knockout mice and insensitivity to $[Ca^{2+}]_c$ stimulation of O_2 consumption in mice.

4.2 Cytosolic Ca^{2+} Buffering

Apart from mitochondria's role as the main energy supplier, its implication in cytosolic Ca^{2+} buffering is becoming increasingly apparent. Mitochondria can directly influence the $[Ca^{2+}]_c$ by importing Ca^{2+} through the MCU and efflux through the Na^+/Ca^{2+} exchanger or H^+/Ca^{2+} exchangers (Gunter et al. 1994; Gunter and Pfeiffer 1990; Carafoli 1987; Thayer and Miller 1990; Cox and Matlib 1993). Since the resting $[Ca^{2+}]_c$ values are ~100 nM and the $\Delta\Psi_m$ is ~−180 mV, the prediction is that at electrochemical equilibrium, theoretical $[Ca^{2+}]_m$ values could be higher than 0.1 M (Pozzan et al. 2000). However, the low affinity of the MCU to Ca^{2+} (K_d around 10–50 μM), the presence of mitochondrial efflux mechanisms, and the decrease of $\Delta\Psi_m$ upon the cation influx would avert the attainment of

electrochemical equilibrium. Therefore, particularly under resting conditions, mitochondria may not uptake any Ca^{2+}. Based on these considerations, the evident discrepancy between the low affinity of MCU, the low concentration of global cytosolic Ca^{2+} signals, and the amplitude of $[Ca^{2+}]_m$ rises were resolved in the 1990s by the concept of a microdomain of high $[Ca^{2+}]_c$ between ER/SR and mitochondria contact areas (Rizzuto et al. 1993, 1998). According to which, mitochondria are strategically located in close proximity to ER/SR through tethering proteins (Csordas et al. 2006), and these close contact sites provide mitochondria preferential access to a much higher $[Ca^{2+}]$ than that measured in the bulk cytosol during Ca^{2+} release from ER/SR and able to activate the MCU. These local $[Ca^{2+}]$ exposures of the mitochondrial surface have been measured to be ~10 μM in average by means of "hotspot" mapping of OMM-targeted Ca^{2+} sensor proteins (Giacomello et al. 2010) or Ca^{2+} sensors directly targeted to the SR/ER-OMM focal contact areas utilizing a drug-inducible heterodimerization strategy (Csordas et al. 2010). In addition, there are reports that VDAC in the OMM and IP3 receptors in the ER are enriched at the mitochondria–ER interface, facilitating a Ca^{2+} transfer from the ER to the mitochondria (Szabadkai et al. 2006; Mendes et al. 2005; Malli et al. 2005). Several functional and morphological studies further suggested that mitochondria can form close contacts not only with ER/SR (Rizzuto et al. 1998; Csordas et al. 1999; Szalai et al. 2000) but also the Golgi apparatus (Dolman et al. 2005) and the plasma membrane (Malli et al. 2003; Park et al. 2001; Varadi et al. 2004). However, among these interactions, the ER/SR-mitochondria connections have gained much attention, and various proteins have been proposed to link mitochondria to the ER/SR such as MIRO, MFN2, and the Mmm1/Mdm10/Mdm12/Mdm34 complex (Rowland and Voeltz 2012; Grimm 2012). Therefore, ER/SR-mitochondria communication also serves as a highly localized Ca^{2+} buffering system. This in turn can modify the activity of any nearby Ca^{2+}-dependent proteins. Such regulation has been reported for IP_3R that display isoform-specific biphasic dependence on $[Ca^{2+}]_c$. Depending on the dominating IP_3R isoform, local Ca^{2+} clearance by mitochondria can either suppress IP_3R activation (and Ca^{2+} release from the ER) via reducing the local $[Ca^{2+}]$ (and so IP_3 sensitivity) over IP_3R clusters (Marchant et al. 2002; Hajnoczky et al. 1999); or do the opposite by decreasing $[Ca^{2+}]$ from high inhibitory to stimulatory range (Olson et al. 2010). By similar principles, local mitochondrial Ca^{2+} clearance has also been implicated in sustaining the activation of I_{CRAC}/Orai channels during store operated Ca^{2+} entry by relieving local feedback inhibition of the channels by Ca^{2+} (Hoth et al. 1997, 2000; Quintana et al. 2006).

4.3 Reactive Oxygen Species Generation

Mitochondria are a major source of ROS in the cell. It has been well recognized that $[Ca^{2+}]_m$ enhance ROS generation by stimulating the TCA cycle and oxidative phosphorylation (Perez-Campo et al. 1998; Sohal and Allen 1985) and/or triggering opening of mPTP (Brookes et al. 2004; Rasola and Bernardi 2011), which plays an

important role in the regulation of cellular function. For example, a recent study identified that mtCU-mediated mitochondrial Ca^{2+} uptake triggers mitochondrial ROS production and transient opening of the mPTP, which promotes wound repair and organismal survival (Xu and Chisholm 2014). In addition, it has been shown that mitochondrial Ca^{2+}-mediated ROS production modulates neural differentiation through activation of the Wnt/β-catenin pathway (Rharass et al. 2014). However, excess Ca^{2+} uptake by the mtCU can be detrimental for cells, triggering excessive ROS generation and initiating cell death pathways such as apoptosis (Mallilankaraman et al. 2012a; Huang et al. 2013; Csordas et al. 2013; Hall et al. 2014). Therefore, mitochondrial Ca^{2+} uptake can be either beneficial or detrimental depending on the amount of Ca^{2+} uptake and cellular conditions. We will discuss the pathological role of mitochondrial Ca^{2+} uptake in Sect. 5.

5 Pathological Roles of Mitochondrial Ca^{2+} Uptake

As shown in Sect. 4.2, mitochondrial Ca^{2+} uptake significantly contributes to buffering cytosolic Ca^{2+} under physiological Ca^{2+} release from ER/SR. However, intensive long-lasting pathophysiological release of Ca^{2+} from ER/SR causes persistent mitochondrial Ca^{2+} accumulation, which consequently triggers excessive ROS generation followed by ATP depletion, the long-lasting opening of the mPTP (Bernardi 2013; Rizzuto et al. 2012), and apoptotic/necrotic cascade (Rizzuto et al. 2012). Accordingly, MCU-overexpressing and MICU1-knockdown human cell lines lead to increased sensitivity to apoptosis (De Stefani et al. 2011; Mallilankaraman et al. 2012a). Moreover, human genetic disease associated with MICU1 null mutations exhibiting central nervous system (extrapyramidal symptoms, learning difficulties) and skeletal muscle (fatigue) phenotypes have been recently identified (Logan et al. 2014; Lewis-Smith et al. 2016). Liver-specific knockout of MICU1 has been recently shown to severely impair liver regeneration after partial hepatectomy, which phenotype could be almost completely rescued by administration of NIM811, a non-immunosuppressant mPTP inhibitor (Antony et al. 2016). In addition, MCU overexpression in *T. brucei* is also sensitized to apoptotic stress (Huang et al. 2013). However, MCU overexpression in a human breast adenocarcinoma cell line (Hall et al. 2014) and MCU-knockout mouse embryonic fibroblasts (Pan et al. 2013) show no difference in sensitivity to apoptosis.

As discussed above, although, mitochondrial Ca^{2+} increase has been associated with apoptosis in many pathological conditions (Giorgi et al. 2012); however, very little is known about the roles of mitochondrial Ca^{2+} signaling in cancer. Marchi et al. (2013) showed that microRNA-25 (miR-25) expression can decrease in MCU gene expression and activity. Specifically, miR-25 is up-regulated in human colon and prostate cancers, which leads to decreased MCU levels followed by reduced mitochondrial Ca^{2+} uptake and resistance to Ca^{2+}-dependent apoptotic challenges (Marchi et al. 2013). Consistent with these results, overexpression of MCU or knockdown of MICU1 in HeLa cervical cancer cells results in constitutive mitochondrial Ca^{2+} influx and increases HeLa cell sensitivity to hydrogen peroxide and

ceramide toxicity (De Stefani et al. 2011; Mallilankaraman et al. 2012a). In other cancer paradigms like in triple-negative breast cancer MCU has been identified as a promoter of progression/invasiveness by supporting the mitochondrial Ca^{2+}-ROS-HIF-1α signaling axis (Tosatto et al. 2016). Thus, the suppression of the MCU expression by miRNA provides initial clues to the relevance of this pathway in human cancers.

Recent studies show that genetic and molecular manipulation of the mtCU complex can also affect cell-type specific functions such as neurotransmission, growth, and development. MCU overexpression increases NMDA receptor-dependent excitotoxicity in mouse neurons via enhanced mitochondrial calcium uptake resulting in aggravated mitochondrial depolarization and neuronal injury. However, MCU knockdown protects neurons against NMDA receptor-mediated excitotoxic cell death (Qiu et al. 2013).

6 Pharmacological Modulators of the MCU

Despite the well-known role of the MCU as a key controller of Ca^{2+} homoeostasis, there is little information about its pharmacological regulation. Although, several pharmacological inhibitors have been described to modify the activity of the MCU, their lack of specificity and cellular permeability has limited their application (Table 1). One of the most widely studied and effective inhibitors is the hexavalent polysaccharide stain, ruthenium red, or its derivate Ru360 (Kirichok et al. 2004; Matlib et al. 1998). In 2011, De Stefani et al. demonstrated the MCU role as the channel-forming subunit, permeable to Ca^{2+} and inhibited by ruthenium red, in an isolated mitochondria. They reconstituted MCU in lipid bilayers and recorded

Table 1 Pharmacological modulators of MCU

Compound	Effect(s)	References
Ruthenium compound: ruthenium red, Ru360	Inhibitor	Kirichok et al. (2004) and Matlib et al. (1998)
Lanthanides: La^{3+}, Gd^{3+}, and Pr^{3+}	Inhibitor	Crompton et al. (1979)
Cardioactive drugs: quinidine, alprenolol, propranolol, oxyfedrine, and tetracaine	Inhibitor	Noack and Greeff (1971)
Amiloride analogs and derivatives	Inhibitor	Schellenberg et al. (1985)
Mg^{2+}	Inhibitor	Szanda et al. (2009)
KBR7943	Inhibitor	Santo-Domingo et al. (2007)
Minocycline	Inhibitor	Schwartz et al. (2013) and Csordas et al. (2012)
Polyamines: spermine and spermidine	Activator	Salvi and Toninello (2004)
Estrogen receptor agonists: 4,4′,4″-(4-propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol (PPT)	Activator	Lobaton et al. (2005)
The p38 MAP kinase inhibitor: SB202190	Activator	Montero et al. (2002)
Flavonoids	Activator	Montero et al. (2004)

ruthenium red-sensitive Ca^{2+} current with 6–7-pS single-channel activity (De Stefani et al. 2011). These findings were very recently supported by another patch-clamp experiment by Chaudhuri et al. (2013). They showed parallel changes in the mitochondrial Ca^{2+} current in an MCU knockdown and overexpression system. In addition, by exploiting the inhibitory characteristic of ruthenium red they further confirmed MCU as a pore-forming subunit of the channel complex. They demonstrated that a single point mutation (S259A) in the putative pore domain conferred resistance to ruthenium red (Baughman et al. 2011; Chaudhuri et al. 2013) without changing current magnitude indicating that ruthenium red directly targets the channel.

However, ruthenium red binds to and inhibits a wide variety of plasma membrane and intracellular Ca^{2+} and K^+ channels like Transient Receptor Potential Vanilloid (TRPV) (Amann and Maggi 1991; Hymel et al. 1988), TWIK-related Acid-sensitive K^+ channel (TASK-3) (Czirjak and Enyedi 2002), and RyR (MacQuaide et al. 2010). Ru360, a purified form of ruthenium red, is more effective than ruthenium red with an IC_{50} 5 nM vs 1 μM, respectively (Ying et al. 1991). Ru360 also demonstrates better specificity for the MCU over other Ca^{2+} channels in cardiac muscles (De Stefani et al. 2011; Baughman et al. 2011; Matlib et al. 1998). Earlier studies have reported a number of drugs exhibiting MCU inhibition such as the cardioactive drugs quinidine, alprenolol, propranolol, oxyfedrine, tetracaine (Noack and Greeff 1971), the diuretic, ethacrynic acid, amiloride analogs and derivatives (Schellenberg et al. 1985), and the antibiotic gentamicin (Sastrasinh et al. 1982). Minocycline, a tetracycline-derived antibiotic that has been used clinically to treat bacterial infections, is also a potent inhibitor for MCU (Schwartz et al. 2013). Mg^{2+}, an antagonist of mitochondrial Ca^{2+} uptake also inhibits the MCU at physiological concentrations (Szanda et al. 2009). Lanthanides such as La^{3+}, Gd^{3+}, and Pr^{3+} are also well-known competitive inhibitors and at low concentrations they may activate the uniporter's activation site and facilitate the transport of other ions (Mela 1969). However, they inhibit a variety of other Ca^{2+} channels and pumps too. Thiourea derivate KBR7943, originally an inhibitor of the plasma membrane Na^+/Ca^{2+} exchanger is also reported to have an inhibitory effect on the MCU (Santo-Domingo et al. 2007). In addition, MCU activity is also inhibited by adenine nucleotides; ATP being the most potent inhibitor (EC_{50} 0.6 mM) followed by ADP > AMP. Interestingly, AMPPNP, a non-hydrolysable analog of ATP was also found to be as efficient as ATP, suggesting that inhibitory action does not require ATP hydrolysis (Litsky and Pfeiffer 1997). On the other hand, uniporter activity is known to be activated by inorganic phosphate (Pi), which can accelerate the Ca^{2+} uptake rate by precipitating with Ca^{2+} in the mitochondrial matrix, and thereby lowering the $[Ca^{2+}]_m$ (Crompton et al. 1983). The Ca^{2+} influx rate and affinity for Ca^{2+} are modulated by protein kinases. Specifically, the ζ isoform of protein kinase C will activate, whereas the β/δ isoforms inactivate MCU (Pinton et al. 2004). Knockdown studies of p38 mitogen-activated protein kinase (MAPK) have resulted in an increase of mitochondrial Ca^{2+} uptake suggesting either itself or its downstream targets can inhibit MCU (Koncz et al. 2009; Szanda et al. 2008). Likewise, SB202190, an inhibitor of p38 MAPK, significantly activates mitochondrial Ca^{2+} uptake, both in

intact and in permeabilized cells (Montero et al. 2002). Other pharmacological activators include natural plant flavonoids (e.g., genistein, quercetin, kaempferol) (Montero et al. 2004), polyamines such as spermine and spermidine (Nicchitta and Williamson 1984; Salvi and Toninello 2004), and estrogens receptor agonists [4,4′,4″-(4-propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol (PPT)] (Lobaton et al. 2005). Lastly, MCU mediated Ca^{2+} uptake also displays allosteric positive regulation by cytosolic Ca^{2+} in a calmodulin-dependent manner (Moreau et al. 2006; Putney and Thomas 2006) which was shown to be inhibited by calmodulin inhibitors (Csordas and Hajnoczky 2003).

7 Conclusions

Ca^{2+} uptake into the mitochondrial matrix plays a vital role in the regulation of multiple physiological and pathological processes, ranging from cytoplasmic Ca^{2+} signaling to bioenergetics and cell death. Mitochondria can uptake Ca^{2+} via multiple channels and pathways, however, the mtCU complex is the most prominent and well-characterized pathway. In this chapter, we have focused on the recent identification of the components of the mtCU complex as well as the other mitochondrial ion channels. Our understanding about the molecular complexity of mtCU gradually evolved from the concept of a single protein to macromolecular signaling complexes, which includes a Ca^{2+} pore-forming component and regulatory components controlling channel activity. We discussed the means by which multiple cell types and tissues regulate and use these channels to best-function for their physiological role in an organism, as well as how the dysfunction of this system can lead to pathophysiological conditions.

The recent characterization of the mtCU complex has opened up the possibility for precise crystal and cryo-electronmicroscopic structural information of the individual proteins as well as the complete complex. Finally, future insight into the transcriptional, post-transcriptional, and post-translational modifications of the multi-protein mtCU complex as well as other mitochondrial Ca^{2+} transport mechanisms will contribute to the development of more specific pharmacological tools and potentially therapeutic drugs.

Acknowledgments This work was partly supported by National Heart, Lung, and Blood Institute Grants 2R01 HL-093671 and HL-114760 (to S. S. Sheu), 1R01 HL-122124 (to G. Csordas and S. S. Sheu). American Heart Association Grant 16SDG27260248 (to J. O-Uchi).

References

Alam MR, Groschner LN, Parichatikanond W, Kuo L, Bondarenko AI, Rost R, Waldeck-Weiermair M, Malli R, Graier WF (2012) Mitochondrial Ca2+ uptake 1 (MICU1) and mitochondrial Ca2+ uniporter (MCU) contribute to metabolism-secretion coupling in clonal pancreatic beta-cells. J Biol Chem 287:34445–34454

Amann R, Maggi CA (1991) Ruthenium red as a capsaicin antagonist. Life Sci 49:849–856

Antony AN, Paillard M, Moffat C, Juskeviciute E, Correnti J, Bolon B, Rubin E, Csordas G, Seifert EL, Hoek JB, Hajnoczky G (2016) MICU1 regulation of mitochondrial Ca(2+) uptake dictates survival and tissue regeneration. Nat Commun 7:10955

Bathori G, Csordas G, Garcia-Perez C, Davies E, Hajnoczky G (2006) Ca2+-dependent control of the permeability properties of the mitochondrial outer membrane and voltage-dependent anion-selective channel (VDAC). J Biol Chem 281:17347–17358

Baughman JM, Perocchi F, Girgis HS, Plovanich M, Belcher-Timme CA, Sancak Y, Bao XR, Strittmatter L, Goldberger O, Bogorad RL, Koteliansky V, Mootha VK (2011) Integrative genomics identifies mcu as an essential component of the mitochondrial calcium uniporter. Nature 476:341–345

Becker GL, Fiskum G, Lehninger AL (1980) Regulation of free Ca2+ by liver mitochondria and endoplasmic reticulum. J Biol Chem 255:9009–9012

Bernardi P (2013) The mitochondrial permeability transition pore: a mystery solved? Front Physiol 4:95

Beutner G, Sharma VK, Giovannucci DR, Yule DI, Sheu SS (2001) Identification of a ryanodine receptor in rat heart mitochondria. J Biol Chem 276:21482–21488

Beutner G, Sharma VK, Lin L, Ryu SY, Dirksen RT, Sheu SS (2005) Type 1 ryanodine receptor in cardiac mitochondria: transducer of excitation-metabolism coupling. Biochim Biophys Acta 1717:1–10

Boerries M, Most P, Gledhill JR, Walker JE, Katus HA, Koch WJ, Aebi U, Schoenenberger CA (2007) Ca2+-dependent interaction of s100a1 with f1-atpase leads to an increased atp content in cardiomyocytes. Mol Cell Biol 27:4365–4373

Bogeski I, Gulaboski R, Kappl R, Mirceski V, Stefova M, Petreska J, Hoth M (2011) Calcium binding and transport by coenzyme Q. J Am Chem Soc 133:9293–9303

Brookes PS, Yoon Y, Robotham JL, Anders MW, Sheu SS (2004) Calcium, ATP, and ROS: a mitochondrial love-hate triangle. Am J Physiol Cell Physiol 287:C817–C833

Buntinas L, Gunter KK, Sparagna GC, Gunter TE (2001) The rapid mode of calcium uptake into heart mitochondria (RaM): comparison to RaM in liver mitochondria. Biochim Biophys Acta 1504:248–261

Carafoli E (1987) Intracellular calcium homeostasis. Annu Rev Biochem 56:395–433

Carafoli E (2010) The fateful encounter of mitochondria with calcium: how did it happen? Biochim Biophys Acta 1797:595–606

Chance B (1965) The energy-linked reaction of calcium with mitochondria. J Biol Chem 240:2729–2748

Chaudhuri D, Sancak Y, Mootha VK, Clapham DE (2013) MCU encodes the pore conducting mitochondrial calcium currents. Elife 2:e00704

Chaudhuri D, Artiga DJ, Abiria SA, Clapham DE (2016) Mitochondrial calcium uniporter regulator 1 (MCUR1) regulates the calcium threshold for the mitochondrial permeability transition. Proc Natl Acad Sci U S A 113:E1872–E1880

Cox DA, Matlib MA (1993) Modulation of intramitochondrial free Ca2+ concentration by antagonists of Na(+)-Ca2+ exchange. Trends Pharmacol Sci 14:408–413

Crompton M, Heid I, Baschera C, Carafoli E (1979) The resolution of calcium fluxes in heart and liver mitochondria using the lanthanide series. FEBS Lett 104:352–354

Crompton M, Kessar P, Al-Nasser I (1983) The alpha-adrenergic-mediated activation of the cardiac mitochondrial Ca2+ uniporter and its role in the control of intramitochondrial Ca2+ in vivo. Biochem J 216:333–342

Csordas G, Hajnoczky G (2003) Plasticity of mitochondrial calcium signaling. J Biol Chem 278:42273–42282

Csordas G, Thomas AP, Hajnoczky G (1999) Quasi-synaptic calcium signal transmission between endoplasmic reticulum and mitochondria. EMBO J 18:96–108

Csordas G, Madesh M, Antonsson B, Hajnoczky G (2002) Tcbid promotes Ca(2+) signal propagation to the mitochondria: control of Ca(2+) permeation through the outer mitochondrial membrane. EMBO J 21:2198–2206

Csordas G, Renken C, Varnai P, Walter L, Weaver D, Buttle KF, Balla T, Mannella CA, Hajnoczky G (2006) Structural and functional features and significance of the physical linkage between ER and mitochondria. J Cell Biol 174:915–921

Csordas G, Varnai P, Golenar T, Roy S, Purkins G, Schneider TG, Balla T, Hajnoczky G (2010) Imaging interorganelle contacts and local calcium dynamics at the ER-mitochondrial interface. Mol Cell 39:121–132

Csordas G, Varnai P, Golenar T, Sheu SS, Hajnoczky G (2012) Calcium transport across the inner mitochondrial membrane: molecular mechanisms and pharmacology. Mol Cell Endocrinol 353:109–113

Csordas G, Golenar T, Seifert EL, Kamer KJ, Sancak Y, Perocchi F, Moffat C, Weaver D, de la Fuente Perez S, Bogorad R, Koteliansky V, Adijanto J, Mootha VK, Hajnoczky G (2013) MICU1 controls both the threshold and cooperative activation of the mitochondrial Ca(2)(+) uniporter. Cell Metab 17:976–987

Czirjak G, Enyedi P (2002) Formation of functional heterodimers between the task-1 and task-3 two-pore domain potassium channel subunits. J Biol Chem 277:5426–5432

de la Fuente S, Matesanz-Isabel J, Fonteriz RI, Montero M, Alvarez J (2014) Dynamics of mitochondrial Ca2+ uptake in MICU1-knockdown cells. Biochem J 458:33–40

De Stefani D, Raffaello A, Teardo E, Szabo I, Rizzuto R (2011) A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter. Nature 476:336–340

Deluca HF, Engstrom GW (1961) Calcium uptake by rat kidney mitochondria. Proc Natl Acad Sci U S A 47:1744–1750

Denton RM (2009) Regulation of mitochondrial dehydrogenases by calcium ions. Biochim Biophys Acta 1787:1309–1316

Denton RM, McCormack JG (1980) The role of calcium in the regulation of mitochondrial metabolism. Biochem Soc Trans 8:266–268

Denton RM, Randle PJ, Martin BR (1972) Stimulation by calcium ions of pyruvate dehydrogenase phosphate phosphatase. Biochem J 128:161–163

Dolman NJ, Gerasimenko JV, Gerasimenko OV, Voronina SG, Petersen OH, Tepikin AV (2005) Stable golgi-mitochondria complexes and formation of golgi Ca(2+) gradients in pancreatic acinar cells. J Biol Chem 280:15794–15799

Doonan PJ, Chandramoorthy HC, Hoffman NE, Zhang X, Cardenas C, Shanmughapriya S, Rajan S, Vallem S, Chen X, Foskett JK, Cheung JY, Houser SR, Madesh M (2014) Letm1-dependent mitochondrial Ca2+ flux modulates cellular bioenergetics and proliferation. FASEB J 28:4936–4949

Drago I, De Stefani D, Rizzuto R, Pozzan T (2012) Mitochondrial Ca2+ uptake contributes to buffering cytoplasmic Ca2+ peaks in cardiomyocytes. Proc Natl Acad Sci U S A 109:12986–12991

Duchen MR, Verkhratsky A, Muallem S (2008) Mitochondria and calcium in health and disease. Cell Calcium 44:1–5

Feng S, Li H, Tai Y, Huang J, Su Y, Abramowitz J, Zhu MX, Birnbaumer L, Wang Y (2013) Canonical transient receptor potential 3 channels regulate mitochondrial calcium uptake. Proc Natl Acad Sci U S A 110:11011–11016

Fieni F, Lee SB, Jan YN, Kirichok Y (2012) Activity of the mitochondrial calcium uniporter varies greatly between tissues. Nat Commun 3:1317

Fieni F, Johnson DE, Hudmon A, Kirichok Y (2014) Mitochondrial Ca2+ uniporter and camkii in heart. Nature 513:E1–E2

Giacomello M, Drago I, Pizzo P, Pozzan T (2007) Mitochondrial Ca2+ as a key regulator of cell life and death. Cell Death Differ 14:1267–1274

Giacomello M, Drago I, Bortolozzi M, Scorzeto M, Gianelle A, Pizzo P, Pozzan T (2010) Ca2+ hot spots on the mitochondrial surface are generated by Ca2+ mobilization from stores, but not by activation of store-operated Ca2+ channels. Mol Cell 38:280–290

Giannini G, Conti A, Mammarella S, Scrobogna M, Sorrentino V (1995) The ryanodine receptor/calcium channel genes are widely and differentially expressed in murine brain and peripheral tissues. J Cell Biol 128:893–904

Giorgi C, Baldassari F, Bononi A, Bonora M, De Marchi E, Marchi S, Missiroli S, Patergnani S, Rimessi A, Suski JM, Wieckowski MR, Pinton P (2012) Mitochondrial Ca(2+) and apoptosis. Cell Calcium 52:36–43

Glancy B, Balaban RS (2012) Role of mitochondrial ca2+ in the regulation of cellular energetics. Biochemistry 51:2959–2973

Grimm S (2012) The ER-mitochondria interface: the social network of cell death. Biochim Biophys Acta 1823:327–334

Gunter TE, Gunter KK (2001) Uptake of calcium by mitochondria: transport and possible function. IUBMB Life 52:197–204

Gunter TE, Pfeiffer DR (1990) Mechanisms by which mitochondria transport calcium. Am J Physiol 258:C755–C786

Gunter TE, Gunter KK, Sheu SS, Gavin CE (1994) Mitochondrial calcium transport: physiological and pathological relevance. Am J Physiol 267:C313–C339

Hajnoczky G, Robb-Gaspers LD, Seitz MB, Thomas AP (1995) Decoding of cytosolic calcium oscillations in the mitochondria. Cell 82:415–424

Hajnoczky G, Hager R, Thomas AP (1999) Mitochondria suppress local feedback activation of inositol 1,4, 5-trisphosphate receptors by Ca2+. J Biol Chem 274:14157–14162

Hall DD, Wu Y, Domann FE, Spitz DR, Anderson ME (2014) Mitochondrial calcium uniporter activity is dispensable for MDA-MB-231 breast carcinoma cell survival. PLoS One 9:e96866

Harrington JL, Murphy E (2015) The mitochondrial calcium uniporter: mice can live and die without it. J Mol Cell Cardiol 78:46–53

Hoffman NE, Chandramoorthy HC, Shamugapriya S, Zhang X, Rajan S, Mallilankaraman K, Gandhirajan RK, Vagnozzi RJ, Ferrer LM, Sreekrishnanilayam K, Natarajaseenivasan K, Vallem S, Force T, Choi ET, Cheung JY, Madesh M (2013) MICU1 motifs define mitochondrial calcium uniporter binding and activity. Cell Rep 5:1576–1588

Holmstrom KM, Pan X, Liu JC, Menazza S, Liu J, Nguyen TT, Pan H, Parks RJ, Anderson S, Noguchi A, Springer D, Murphy E, Finkel T (2015) Assessment of cardiac function in mice lacking the mitochondrial calcium uniporter. J Mol Cell Cardiol 85:178–182

Hoth M, Fanger CM, Lewis RS (1997) Mitochondrial regulation of store-operated calcium signaling in T lymphocytes. J Cell Biol 137:633–648

Hoth M, Button DC, Lewis RS (2000) Mitochondrial control of calcium-channel gating: a mechanism for sustained signaling and transcriptional activation in T lymphocytes. Proc Natl Acad Sci U S A 97:10607–10612

Huang G, Vercesi AE, Docampo R (2013) Essential regulation of cell bioenergetics in trypanosoma brucei by the mitochondrial calcium uniporter. Nat Commun 4:2865

Hung V, Zou P, Rhee HW, Udeshi ND, Cracan V, Svinkina T, Carr SA, Mootha VK, Ting AY (2014) Proteomic mapping of the human mitochondrial intermembrane space in live cells via ratiometric apex tagging. Mol Cell 55:332–341

Hymel L, Schindler H, Inui M, Fleischer S (1988) Reconstitution of purified cardiac muscle calcium release channel (ryanodine receptor) in planar bilayers. Biochem Biophys Res Commun 152:308–314

Jakob R, Beutner G, Sharma VK, Duan Y, Gross RA, Hurst S, Jhun BS, O-Uchi J, Sheu SS (2014) Molecular and functional identification of a mitochondrial ryanodine receptor in neurons. Neurosci Lett 575:7–12

Jeyakumar LH, Gleaves LA, Ridley BD, Chang P, Atkinson J, Barnett JV, Fleischer S (2002) The skeletal muscle ryanodine receptor isoform 1 is found at the intercalated discs in human and mouse hearts. J Muscle Res Cell Motil 23:285–292

Jiang D, Zhao L, Clapham DE (2009) Genome-wide RNAi screen identifies Letm1 as a mitochondrial Ca2+/H+ antiporter. Science 326:144–147

Joiner ML, Koval OM, Li J, He BJ, Allamargot C, Gao Z, Luczak ED, Hall DD, Fink BD, Chen B, Yang J, Moore SA, Scholz TD, Strack S, Mohler PJ, Sivitz WI, Song LS, Anderson ME (2012) Camkii determines mitochondrial stress responses in heart. Nature 491:269–273

Joiner ML, Koval OM, Li J, He BJ, Allamargot C, Gao Z, Luczak ED, Hall DD, Fink BD, Chen B, Yang J, Moore SA, Scholz TD, Strack S, Mohler PJ, Sivitz WI, Song LS, Anderson ME, Joiner et al (2014) Reply. Nature 513:E3

Jouaville LS, Pinton P, Bastianutto C, Rutter GA, Rizzuto R (1999) Regulation of mitochondrial atp synthesis by calcium: evidence for a long-term metabolic priming. Proc Natl Acad Sci U S A 96:13807–13812

Kamer KJ, Mootha VK (2014) MICU1 and MICU2 play nonredundant roles in the regulation of the mitochondrial calcium uniporter. EMBO Rep 15:299–307

Kirichok Y, Krapivinsky G, Clapham DE (2004) The mitochondrial calcium uniporter is a highly selective ion channel. Nature 427:360–364

Koncz P, Szanda G, Fulop L, Rajki A, Spat A (2009) Mitochondrial Ca2+ uptake is inhibited by a concerted action of p38 mapk and protein kinase D. Cell Calcium 46:122–129

Kovacs-Bogdan E, Sancak Y, Kamer KJ, Plovanich M, Jambhekar A, Huber RJ, Myre MA, Blower MD, Mootha VK (2014) Reconstitution of the mitochondrial calcium uniporter in yeast. Proc Natl Acad Sci U S A 111:8985–8990

Kwong JQ, Lu X, Correll RN, Schwanekamp JA, Vagnozzi RJ, Sargent MA, York AJ, Zhang J, Bers DM, Molkentin JD (2015) The mitochondrial calcium uniporter selectively matches metabolic output to acute contractile stress in the heart. Cell Rep 12:15–22

Lam SS, Martell JD, Kamer KJ, Deerinck TJ, Ellisman MH, Mootha VK, Ting AY (2015) Directed evolution of apex2 for electron microscopy and proximity labeling. Nat Methods 12:51–54

Lanner JT, Georgiou DK, Joshi AD, Hamilton SL (2010) Ryanodine receptors: structure, expression, molecular details, and function in calcium release. Cold Spring Harb Perspect Biol 2: a003996

Lee Y, Min CK, Kim TG, Song HK, Lim Y, Kim D, Shin K, Kang M, Kang JY, Youn HS, Lee JG, An JY, Park KR, Lim JJ, Kim JH, Kim JH, Park ZY, Kim YS, Wang J, do Kim H, Eom SH (2015) Structure and function of the n-terminal domain of the human mitochondrial calcium uniporter. EMBO Rep 16:1318–1333

Lee SK, Shanmughapriya S, Mok MC, Dong Z, Tomar D, Carvalho E, Rajan S, Junop MS, Madesh M, Stathopulos PB (2016) Structural insights into mitochondrial calcium uniporter regulation by divalent cations. Cell Chem Biol 23:1157–1169

Lewis-Smith D, Kamer KJ, Griffin H, Childs AM, Pysden K, Titov D, Duff J, Pyle A, Taylor RW, Yu-Wai-Man P, Ramesh V, Horvath R, Mootha VK, Chinnery PF (2016) Homozygous deletion in MICU1 presenting with fatigue and lethargy in childhood. Neurol Genet 2:e59

Litsky ML, Pfeiffer DR (1997) Regulation of the mitochondrial Ca2+ uniporter by external adenine nucleotides: the uniporter behaves like a gated channel which is regulated by nucleotides and divalent cations. Biochemistry 36:7071–7080

Lobaton CD, Vay L, Hernandez-Sanmiguel E, Santodomingo J, Moreno A, Montero M, Alvarez J (2005) Modulation of mitochondrial Ca(2+) uptake by estrogen receptor agonists and antagonists. Br J Pharmacol 145:862–871

Logan CV, Szabadkai G, Sharpe JA, Parry DA, Torelli S, Childs AM, Kriek M, Phadke R, Johnson CA, Roberts NY, Bonthron DT, Pysden KA, Whyte T, Munteanu I, Foley AR, Wheway G, Szymanska K, Natarajan S, Abdelhamed ZA, Morgan JE, Roper H, Santen GW, Niks EH, van der Pol WL, Lindhout D, Raffaello A, De Stefani D, den Dunnen JT, Sun Y, Ginjaar I, Sewry CA, Hurles M, Rizzuto R, Consortium UK, Duchen MR, Muntoni F, Sheridan E (2014) Loss-of-function mutations in MICU1 cause a brain and muscle disorder linked to primary alterations in mitochondrial calcium signaling. Nat Genet 46:188–193

Luongo TS, Lambert JP, Yuan A, Zhang X, Gross P, Song J, Shanmughapriya S, Gao E, Jain M, Houser SR, Koch WJ, Cheung JY, Madesh M, Elrod JW (2015) The mitochondrial calcium

uniporter matches energetic supply with cardiac workload during stress and modulates permeability transition. Cell Rep 12:23–34
MacQuaide N, Ramay HR, Sobie EA, Smith GL (2010) Differential sensitivity of Ca(2)+ wave and Ca(2)+ spark events to ruthenium red in isolated permeabilised rabbit cardiomyocytes. J Physiol 588:4731–4742
Malli R, Frieden M, Osibow K, Zoratti C, Mayer M, Demaurex N, Graier WF (2003) Sustained Ca2+ transfer across mitochondria is essential for mitochondrial Ca2+ buffering, sore-operated Ca2+ entry, and Ca2+ store refilling. J Biol Chem 278:44769–44779
Malli R, Frieden M, Trenker M, Graier WF (2005) The role of mitochondria for Ca2+ refilling of the endoplasmic reticulum. J Biol Chem 280:12114–12122
Mallilankaraman K, Doonan P, Cardenas C, Chandramoorthy HC, Muller M, Miller R, Hoffman NE, Gandhirajan RK, Molgo J, Birnbaum MJ, Rothberg BS, Mak DO, Foskett JK, Madesh M (2012a) MICU1 is an essential gatekeeper for MCU-mediated mitochondrial Ca(2+) uptake that regulates cell survival. Cell 151:630–644
Mallilankaraman K, Cardenas C, Doonan PJ, Chandramoorthy HC, Irrinki KM, Golenar T, Csordas G, Madireddi P, Yang J, Muller M, Miller R, Kolesar JE, Molgo J, Kaufman B, Hajnoczky G, Foskett JK, Madesh M (2012b) MCUR1 is an essential component of mitochondrial Ca2+ uptake that regulates cellular metabolism. Nat Cell Biol 14:1336–1343
Marchant JS, Ramos V, Parker I (2002) Structural and functional relationships between Ca2+ puffs and mitochondria in xenopus oocytes. Am J Physiol Cell Physiol 282:C1374–C1386
Marchi S, Lupini L, Patergnani S, Rimessi A, Missiroli S, Bonora M, Bononi A, Corra F, Giorgi C, De Marchi E, Poletti F, Gafa R, Lanza G, Negrini M, Rizzuto R, Pinton P (2013) Downregulation of the mitochondrial calcium uniporter by cancer-related miR-25. Curr Biol 23:58–63
Marks AR, Tempst P, Hwang KS, Taubman MB, Inui M, Chadwick C, Fleischer S, Nadal-Ginard B (1989) Molecular cloning and characterization of the ryanodine receptor/junctional channel complex cDNA from skeletal muscle sarcoplasmic reticulum. Proc Natl Acad Sci U S A 86:8683–8687
Martell JD, Deerinck TJ, Sancak Y, Poulos TL, Mootha VK, Sosinsky GE, Ellisman MH, Ting AY (2012) Engineered ascorbate peroxidase as a genetically encoded reporter for electron microscopy. Nat Biotechnol 30:1143–1148
Matesanz-Isabel J, Arias-del-Val J, Alvarez-Illera P, Fonteriz RI, Montero M, Alvarez J (2016) Functional roles of MICU1 and MICU2 in mitochondrial Ca(2+) uptake. Biochim Biophys Acta 1858:1110–1117
Matlib MA, Zhou Z, Knight S, Ahmed S, Choi KM, Krause-Bauer J, Phillips R, Altschuld R, Katsube Y, Sperelakis N, Bers DM (1998) Oxygen-bridged dinuclear ruthenium amine complex specifically inhibits Ca2+ uptake into mitochondria in vitro and in situ in single cardiac myocytes. J Biol Chem 273:10223–10231
McCormack JG, Halestrap AP, Denton RM (1990) Role of calcium ions in regulation of mammalian intramitochondrial metabolism. Physiol Rev 70:391–425
Mela L (1969) Inhibition and activation of calcium transport in mitochondria. Effect of lanthanides and local anesthetic drugs. Biochemistry 8:2481–2486
Mendes CC, Gomes DA, Thompson M, Souto NC, Goes TS, Goes AM, Rodrigues MA, Gomez MV, Nathanson MH, Leite MF (2005) The type III inositol 1,4,5-trisphosphate receptor preferentially transmits apoptotic Ca2+ signals into mitochondria. J Biol Chem 280:40892–40900
Michels G, Khan IF, Endres-Becker J, Rottlaender D, Herzig S, Ruhparwar A, Wahlers T, Hoppe UC (2009) Regulation of the human cardiac mitochondrial Ca2+ uptake by 2 different voltage-gated Ca2+ channels. Circulation 119:2435–2443
Montero M, Lobaton CD, Moreno A, Alvarez J (2002) A novel regulatory mechanism of the mitochondrial Ca2+ uniporter revealed by the p38 mitogen-activated protein kinase inhibitor sb202190. FASEB J 16:1955–1957

Montero M, Lobaton CD, Hernandez-Sanmiguel E, Santodomingo J, Vay L, Moreno A, Alvarez J (2004) Direct activation of the mitochondrial calcium uniporter by natural plant flavonoids. Biochem J 384:19–24

Moreau B, Nelson C, Parekh AB (2006) Biphasic regulation of mitochondrial Ca2+ uptake by cytosolic Ca2+ concentration. Curr Biol 16:1672–1677

Munch G, Bolck B, Sugaru A, Schwinger RH (2000) Isoform expression of the sarcoplasmic reticulum Ca2+ release channel (ryanodine channel) in human myocardium. J Mol Med 78:352–360

Murgia M, Rizzuto R (2015) Molecular diversity and pleiotropic role of the mitochondrial calcium uniporter. Cell Calcium 58:11–17

Murphy E, Pan X, Nguyen T, Liu J, Holmstrom KM, Finkel T (2014) Unresolved questions from the analysis of mice lacking MCU expression. Biochem Biophys Res Commun 449:384–385

Nakai J, Imagawa T, Hakamat Y, Shigekawa M, Takeshima H, Numa S (1990) Primary structure and functional expression from cdna of the cardiac ryanodine receptor/calcium release channel. FEBS Lett 271:169–177

Nicchitta CV, Williamson JR (1984) Spermine. A regulator of mitochondrial calcium cycling. J Biol Chem 259:12978–12983

Nicholls DG (1978) The regulation of extramitochondrial free calcium ion concentration by rat liver mitochondria. Biochem J 176:463–474

Noack E, Greeff K (1971) Inhibition of calcium transport in mitochondria by -receptor blocking substances and its reactivation by phospholipids. Experientia 27:810–811

Olson ML, Chalmers S, McCarron JG (2010) Mitochondrial Ca2+ uptake increases Ca2+ release from inositol 1,4,5-trisphosphate receptor clusters in smooth muscle cells. J Biol Chem 285:2040–2050

O-Uchi J, Pan S, Sheu SS (2012) Perspectives on: SGP Symposium on Mitochondrial Physiology and Medicine. Molecular identities of mitochondrial Ca^{2+} influx mechanism: updated passwords for accessing mitochondrial Ca^{2+}-linked health and disease. J Gen Physiol 139:435–443

O-Uchi J, Jhun BS, Hurst S, Bisetto S, Gross P, Chen M, Kettlewell S, Park J, Oyamada H, Smith GL, Murayama T, Sheu SS (2013) Overexpression of ryanodine receptor type 1 enhances mitochondrial fragmentation and Ca^{2+}-induced ATP production in cardiac H9c2 myoblasts. Am J Physiol Heart Circ Physiol 305:H1736–H1751

O-Uchi J, Jhun BS, Xu S, Hurst S, Raffaello A, Liu X, Yi B, Zhang H, Gross P, Mishra J, Ainbinder A, Kettlewell S, Smith GL, Dirksen RT, Wang W, Rizzuto R, Sheu SS (2014) Adrenergic signaling regulates mitochondrial Ca2+ uptake through Pyk2-dependent tyrosine phosphorylation of the mitochondrial Ca2+ uniporter. Antioxid Redox Signal 21:863–879

Oxenoid K, Dong Y, Cao C, Cui T, Sancak Y, Markhard AL, Grabarek Z, Kong L, Liu Z, Ouyang B, Cong Y, Mootha VK, Chou JJ (2016) Architecture of the mitochondrial calcium uniporter. Nature 533:269–273

Pagliarini DJ, Calvo SE, Chang B, Sheth SA, Vafai SB, Ong SE, Walford GA, Sugiana C, Boneh A, Chen WK, Hill DE, Vidal M, Evans JG, Thorburn DR, Carr SA, Mootha VK (2008) A mitochondrial protein compendium elucidates complex i disease biology. Cell 134:112–123

Pan X, Liu J, Nguyen T, Liu C, Sun J, Teng Y, Fergusson MM, Rovira II, Allen M, Springer DA, Aponte AM, Gucek M, Balaban RS, Murphy E, Finkel T (2013) The physiological role of mitochondrial calcium revealed by mice lacking the mitochondrial calcium uniporter. Nat Cell Biol 15:1464–1472

Park MK, Ashby MC, Erdemli G, Petersen OH, Tepikin AV (2001) Perinuclear, perigranular and sub-plasmalemmal mitochondria have distinct functions in the regulation of cellular calcium transport. EMBO J 20:1863–1874

Patron M, Checchetto V, Raffaello A, Teardo E, Vecellio Reane D, Mantoan M, Granatiero V, Szabo I, De Stefani D, Rizzuto R (2014) MICU1 and MICU2 finely tune the mitochondrial Ca2+ uniporter by exerting opposite effects on mcu activity. Mol Cell 53:726–737

Paupe V, Prudent J, Dassa EP, Rendon OZ, Shoubridge EA (2015) CCDC90A (MCUR1) is a cytochrome c oxidase assembly factor and not a regulator of the mitochondrial calcium uniporter. Cell Metab 21:109–116

Perez-Campo R, Lopez-Torres M, Cadenas S, Rojas C, Barja G (1998) The rate of free radical production as a determinant of the rate of aging: evidence from the comparative approach. J Comp Physiol B 168:149–158

Perocchi F, Gohil VM, Girgis HS, Bao XR, McCombs JE, Palmer AE, Mootha VK (2010) Micu1 encodes a mitochondrial ef hand protein required for ca(2+) uptake. Nature 467:291–296

Petrungaro C, Zimmermann KM, Kuttner V, Fischer M, Dengjel J, Bogeski I, Riemer J (2015) The Ca(2+)-dependent release of the Mia40-induced MICU1-MICU2 dimer from MCU regulates mitochondrial Ca(2+) uptake. Cell Metab 22:721–733

Pinton P, Leo S, Wieckowski MR, Di Benedetto G, Rizzuto R (2004) Long-term modulation of mitochondrial Ca^{2+} signals by protein kinase C isozymes. J Cell Biol 165:223–232

Plovanich M, Bogorad RL, Sancak Y, Kamer KJ, Strittmatter L, Li AA, Girgis HS, Kuchimanchi S, De Groot J, Speciner L, Taneja N, Oshea J, Koteliansky V, Mootha VK (2013) MICU2, a paralog of MICU1, resides within the mitochondrial uniporter complex to regulate calcium handling. PLoS One 8:e55785

Pozzan T, Magalhaes P, Rizzuto R (2000) The comeback of mitochondria to calcium signalling. Cell Calcium 28:279–283

Putney JW Jr, Thomas AP (2006) Calcium signaling: double duty for calcium at the mitochondrial uniporter. Curr Biol 16:R812–R815

Qiu J, Tan YW, Hagenston AM, Martel MA, Kneisel N, Skehel PA, Wyllie DJ, Bading H, Hardingham GE (2013) Mitochondrial calcium uniporter mcu controls excitotoxicity and is transcriptionally repressed by neuroprotective nuclear calcium signals. Nat Commun 4:2034

Quan X, Nguyen TT, Choi SK, Xu S, Das R, Cha SK, Kim N, Han J, Wiederkehr A, Wollheim CB, Park KS (2015) Essential role of mitochondrial Ca^{2+} uniporter in the generation of mitochondrial pH gradient and metabolism-secretion coupling in insulin-releasing cells. J Biol Chem 290:4086–4096

Quintana A, Schwarz EC, Schwindling C, Lipp P, Kaestner L, Hoth M (2006) Sustained activity of calcium release-activated calcium channels requires translocation of mitochondria to the plasma membrane. J Biol Chem 281:40302–40309

Raffaello A, De Stefani D, Sabbadin D, Teardo E, Merli G, Picard A, Checchetto V, Moro S, Szabo I, Rizzuto R (2013) The mitochondrial calcium uniporter is a multimer that can include a dominant-negative pore-forming subunit. EMBO J 32:2362–2376

Rapizzi E, Pinton P, Szabadkai G, Wieckowski MR, Vandecasteele G, Baird G, Tuft RA, Fogarty KE, Rizzuto R (2002) Recombinant expression of the voltage-dependent anion channel enhances the transfer of Ca2+ microdomains to mitochondria. J Cell Biol 159:613–624

Rasola A, Bernardi P (2011) Mitochondrial permeability transition in Ca(2+)-dependent apoptosis and necrosis. Cell Calcium 50:222–233

Rharass T, Lemcke H, Lantow M, Kuznetsov SA, Weiss DG, Panakova D (2014) Ca2+-mediated mitochondrial reactive oxygen species metabolism augments Wnt/beta-catenin pathway activation to facilitate cell differentiation. J Biol Chem 289:27937–27951

Rizzuto R, Pozzan T (2006) Microdomains of intracellular Ca2+: molecular determinants and functional consequences. Physiol Rev 86:369–408

Rizzuto R, Brini M, Murgia M, Pozzan T (1993) Microdomains with high Ca2+ close to IP3-sensitive channels that are sensed by neighboring mitochondria. Science 262:744–747

Rizzuto R, Pinton P, Carrington W, Fay FS, Fogarty KE, Lifshitz LM, Tuft RA, Pozzan T (1998) Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca2+ responses. Science 280:1763–1766

Rizzuto R, De Stefani D, Raffaello A, Mammucari C (2012) Mitochondria as sensors and regulators of calcium signalling. Nat Rev Mol Cell Biol 13:566–578

Rowland AA, Voeltz GK (2012) Endoplasmic reticulum-mitochondria contacts: function of the junction. Nat Rev Mol Cell Biol 13:607–625

Rutter GA, Denton RM (1988) Regulation of NAD+-linked isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase by Ca2+ ions within toluene-permeabilized rat heart mitochondria. Interactions with regulation by adenine nucleotides and NADH/NAD+ ratios. Biochem J 252:181–189

Ryu SY, Beutner G, Kinnally KW, Dirksen RT, Sheu SS (2011) Single channel characterization of the mitochondrial ryanodine receptor in heart mitoplasts. J Biol Chem 286:21324–21329

Salvi M, Toninello A (2004) Effects of polyamines on mitochondrial Ca(2+) transport. Biochim Biophys Acta 1661:113–124

Sancak Y, Markhard AL, Kitami T, Kovacs-Bogdan E, Kamer KJ, Udeshi ND, Carr SA, Chaudhuri D, Clapham DE, Li AA, Calvo SE, Goldberger O, Mootha VK (2013) EMRE is an essential component of the mitochondrial calcium uniporter complex. Science 342:1379–1382

Santo-Domingo J, Vay L, Hernandez-Sanmiguel E, Lobaton CD, Moreno A, Montero M, Alvarez J (2007) The plasma membrane Na+/Ca2+ exchange inhibitor KB-R7943 is also a potent inhibitor of the mitochondrial Ca2+ uniporter. Br J Pharmacol 151:647–654

Sastrasinh M, Weinberg JM, Humes HD (1982) The effect of gentamicin on calcium uptake by renal mitochondria. Life Sci 30:2309–2315

Schellenberg GD, Anderson L, Cragoe EJ Jr, Swanson PD (1985) Inhibition of brain mitochondrial Ca2+ transport by amiloride analogues. Cell Calcium 6:431–447

Schwartz J, Holmuhamedov E, Zhang X, Lovelace GL, Smith CD, Lemasters JJ (2013) Minocycline and doxycycline, but not other tetracycline-derived compounds, protect liver cells from chemical hypoxia and ischemia/reperfusion injury by inhibition of the mitochondrial calcium uniporter. Toxicol Appl Pharmacol 273:172–179

Seidlmayer LK, Kuhn J, Berbner A, Arias-Loza PA, Williams T, Kaspar M, Czolbe M, Kwong JQ, Molkentin JD, Heinze KG, Dedkova EN, Ritter O (2016) Inositol 1,4,5-trisphosphate-mediated sarcoplasmic reticulum-mitochondrial crosstalk influences adenosine triphosphate production via mitochondrial Ca2+ uptake through the mitochondrial ryanodine receptor in cardiac myocytes. Cardiovasc Res 112:491–501

Shanmughapriya S, Rajan S, Hoffman NE, Zhang X, Guo S, Kolesar JE, Hines KJ, Ragheb J, Jog NR, Caricchio R, Baba Y, Zhou Y, Kaufman BA, Cheung JY, Kurosaki T, Gill DL, Madesh M (2015) Ca2+ signals regulate mitochondrial metabolism by stimulating CREB-mediated expression of the mitochondrial Ca2+ uniporter gene MCU. Sci Signal 8:ra23

Sharma VK, Ramesh V, Franzini-Armstrong C, Sheu SS (2000) Transport of Ca2+ from sarcoplasmic reticulum to mitochondria in rat ventricular myocytes. J Bioenerg Biomembr 32:97–104

Sohal RS, Allen RG (1985) Relationship between metabolic rate, free radicals, differentiation and aging: a unified theory. Basic Life Sci 35:75–104

Sparagna GC, Gunter KK, Sheu SS, Gunter TE (1995) Mitochondrial calcium uptake from physiological-type pulses of calcium. A description of the rapid uptake mode. J Biol Chem 270:27510–27515

Szabadkai G, Bianchi K, Varnai P, De Stefani D, Wieckowski MR, Cavagna D, Nagy AI, Balla T, Rizzuto R (2006) Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca2+ channels. J Cell Biol 175:901–911

Szalai G, Csordas G, Hantash BM, Thomas AP, Hajnoczky G (2000) Calcium signal transmission between ryanodine receptors and mitochondria. J Biol Chem 275:15305–15313

Szanda G, Koncz P, Rajki A, Spat A (2008) Participation of p38 mapk and a novel-type protein kinase C in the control of mitochondrial Ca2+ uptake. Cell Calcium 43:250–259

Szanda G, Rajki A, Gallego-Sandin S, Garcia-Sancho J, Spat A (2009) Effect of cytosolic Mg2+ on mitochondrial Ca2+ signaling. Pflugers Arch Eur J Physiol 457:941–954

Tan W, Colombini M (2007) VDAC closure increases calcium ion flux. Biochim Biophys Acta 1768:2510–2515

Tarasov AI, Semplici F, Ravier MA, Bellomo EA, Pullen TJ, Gilon P, Sekler I, Rizzuto R, Rutter GA (2012) The mitochondrial Ca2+ uniporter mcu is essential for glucose-induced atp increases in pancreatic beta-cells. PLoS One 7:e39722

Thayer SA, Miller RJ (1990) Regulation of the intracellular free calcium concentration in single rat dorsal root ganglion neurones in vitro. J Physiol 425:85–115

Tomar D, Dong Z, Shanmughapriya S, Koch DA, Thomas T, Hoffman NE, Timbalia SA, Goldman SJ, Breves SL, Corbally DP, Nemani N, Fairweather JP, Cutri AR, Zhang X, Song J, Jana F, Huang J, Barrero C, Rabinowitz JE, Luongo TS, Schumacher SM, Rockman ME, Dietrich A, Merali S, Caplan J, Stathopulos P, Ahima RS, Cheung JY, Houser SR, Koch WJ, Patel V, Gohil VM, Elrod JW, Rajan S, Madesh M (2016) MCUR1 is a scaffold factor for the MCU complex function and promotes mitochondrial bioenergetics. Cell Rep 15:1673–1685

Tosatto A, Sommaggio R, Kummerow C, Bentham RB, Blacker TS, Berecz T, Duchen MR, Rosato A, Bogeski I, Szabadkai G, Rizzuto R, Mammucari C (2016) The mitochondrial calcium uniporter regulates breast cancer progression via HIF-1alpha. EMBO Mol Med 8:569–585

Tsai MF, Jiang D, Zhao L, Clapham D, Miller C (2014) Functional reconstitution of the mitochondrial Ca2+/H+ antiporter Letm1. J Gen Physiol 143:67–73

Tsai MF, Phillips CB, Ranaghan M, Tsai CW, Wu Y, Willliams C, Miller C (2016) Dual functions of a small regulatory subunit in the mitochondrial calcium uniporter complex. Elife 5

Vais H, Mallilankaraman K, Mak DO, Hoff H, Payne R, Tanis JE, Foskett JK (2016) EMRE is a matrix Ca(2+) sensor that governs gatekeeping of the mitochondrial Ca(2+) uniporter. Cell Rep 14:403–410

Varadi A, Cirulli V, Rutter GA (2004) Mitochondrial localization as a determinant of capacitative Ca2+ entry in hela cells. Cell Calcium 36:499–508

Vasington FD, Murphy JV (1962) Ca ion uptake by rat kidney mitochondria and its dependence on respiration and phosphorylation. J Biol Chem 237:2670–2677

Vecellio Reane D, Vallese F, Checchetto V, Acquasaliente L, Butera G, De Filippis V, Szabo I, Zanotti G, Rizzuto R, Raffaello A (2016) A MICU1 splice variant confers high sensitivity to the mitochondrial Ca2+ uptake machinery of skeletal muscle. Mol Cell 64:760–773

Wang L, Yang X, Li S, Wang Z, Liu Y, Feng J, Zhu Y, Shen Y (2014) Structural and mechanistic insights into MICU1 regulation of mitochondrial calcium uptake. EMBO J 33:594–604

Wu Y, Rasmussen TP, Koval OM, Joiner ML, Hall DD, Chen B, Luczak ED, Wang Q, Rokita AG, Wehrens XH, Song LS, Anderson ME (2015) The mitochondrial uniporter controls fight or flight heart rate increases. Nat Commun 6:6081

Xu S, Chisholm AD (2014) C. elegans epidermal wounding induces a mitochondrial ros burst that promotes wound repair. Dev Cell 31:48–60

Yamamoto T, Yamagoshi R, Harada K, Kawano M, Minami N, Ido Y, Kuwahara K, Fujita A, Ozono M, Watanabe A, Yamada A, Terada H, Shinohara Y (2016) Analysis of the structure and function of emre in a yeast expression system. Biochim Biophys Acta 1857:831–839

Ying WL, Emerson J, Clarke MJ, Sanadi DR (1991) Inhibition of mitochondrial calcium ion transport by an oxo-bridged dinuclear ruthenium ammine complex. Biochemistry 30:4949–4952

Zimanyi I, Pessah IN (1991) Comparison of [3H]ryanodine receptors and Ca++ release from rat cardiac and rabbit skeletal muscle sarcoplasmic reticulum. J Pharmacol Exp Ther 256:938–946