Am J Physiol Heart Circ Physiol 315: H1341–H1352, 2018.
First published August 10, 2018; doi:10.1152/ajpheart.00028.2018.

REVIEW | *Novel Mechanisms of Myocardial Ischemia, Ischemia-Reperfusion, and Protection by Myocardial Conditioning*

Mitochondrial bioenergetics and cardiolipin alterations in myocardial ischemia-reperfusion injury: implications for pharmacological cardioprotection

Giuseppe Paradies,[1] Valeria Paradies,[3] Francesca Maria Ruggiero,[1] and Giuseppe Petrosillo[2]

[1]*Department of Biosciences, Biotechnologies, and Biopharmaceutics, University of Bari, Bari, Italy;* [2]*Institute of Biomembranes, Bioenergetics, and Molecular Biotechnologies, National Research Council, Bari, Italy; and* [3]*National Heart Centre, Singapore*

Submitted 16 January 2018; accepted in final form 7 August 2018

Paradies G, Paradies V, Ruggiero FM, Petrosillo G. Mitochondrial bioenergetics and cardiolipin alterations in myocardial ischemia-reperfusion injury: implications for pharmacological cardioprotection. *Am J Physiol Heart Circ Physiol* 315: H1341–H1352, 2018. First published August 10, 2018; doi:10.1152/ajpheart.00028.2018.— Mitochondrial dysfunction plays a central role in myocardial ischemia-reperfusion (I/R) injury. Increased reactive oxygen species production, impaired electron transport chain activity, aberrant mitochondrial dynamics, Ca^{2+} overload, and opening of the mitochondrial permeability transition pore have been proposed as major contributory factors to mitochondrial dysfunction during myocardial I/R injury. Cardiolipin (CL), a mitochondria-specific phospholipid, plays a pivotal role in multiple mitochondrial bioenergetic processes, including respiration and energy conversion, in mitochondrial morphology and dynamics as well as in several steps of the apoptotic process. Changes in CL levels, species composition, and degree of oxidation may have deleterious consequences for mitochondrial function with important implications in a variety of pathophysiological conditions, including myocardial I/R injury. In this review, we focus on the role played by CL alterations in mitochondrial dysfunction in myocardial I/R injury. Pharmacological strategies to prevent myocardial injury during I/R targeting mitochondrial CL are also examined.

cardiolipin; cardioprotection; heart ischemia-reperfusion injury; mitochondrial bioenergetics

INTRODUCTION

Myocardial ischemia is widely recognized as a major cause of morbidity and mortality worldwide. This pathological condition occurs because of obstruction of the blood flow through the myocardium and can provoke tissue damage due to lack of oxygen and nutrients required for energy production to drive the heart contractile cycle. It is well known that myocardial ischemia results in loss of contractile function and myocardial damage as a consequence of cell death from both necrosis and apoptosis (30, 45) Timely reperfusion of the ischemic heart restores blood flow, oxygen supply, and nutrients, improving left ventricular postinfarction contractile function and patient survival (33, 109). Although reperfusion after a prolonged period of ischemia is essential to restore normal cellular homeostasis and salvage myocardium, paradoxically this intervention may result in inflammation and oxidative damage through the induction of oxidative stress leading to cardiomyocyte death and subsequent irreversible myocardial injury, a phenomenon commonly called "ischemia-reperfusion (I/R) injury." Much attention has been devoted to characterization of the molecular and cellular basis of the injury response that results when myocardial ischemic tissue is reperfused (58, 71, 84).

It is now widely accepted that mitochondrial dysfunction plays a critical role in determining myocardial I/R injury, and in recent years many reviews have been published on this topic (58, 71, 84, 134). Myocardial I/R is characterized by cardiomyocyte hypercontracture, reduction of left ventricular pressure, augmented vascular resistance, and elevated incidence of ventricular fibrillation. Heart tissue consumes large amounts of energy to maintain contractile activity. To accommodate the high energy requirement to drive the contractile cycle and maintain ionic homeostasis, cardiomyocytes have a high mitochondrial density that comprises ~30% of the total intracellular volume (76). The major role of mitochondria in the heart is the production of ATP through the respiratory metabolism and oxidative phosphorylation (OXPHOS) process. About 90% of cellular ATP produced by cardiomyocytes is used to support the contraction-relaxation cycle within the myocardium. Even subtle alterations in mito-

Address for reprint requests and other correspondence: G. Paradies, Univ. of Bari, via E. Orabona, 4, Bari 70126, Italy (e-mail: g.paradies@biologia.uniba.it).

chondrial function can cause a significant change in cardiomyocyte energy production and further harm cardiovascular health. Mitochondria are intimately involved in the processes that lead to cell death, in both necrotic and apoptotic forms, during heart I/R and are therefore a potential target for cardioprotective interventions (30, 33, 45, 71).

Accumulating evidence suggests that oxidative stress plays a central role in myocardial I/R injury (21, 46, 66, 76, 136). Oxidative stress occurs when the antioxidant system is overwhelmed by overproduction of reactive oxygen species (ROS). Mitochondrial respiration is considered the major site of ROS production, which can be greatly enhanced when mitochondrial respiration is stimulated under conditions of impaired redox state and reduced availability of ADP (4, 15, 21, 61, 66, 71, 108), a condition occurring in heart I/R. Thus, mitochondrial respiration is considered an important source of ROS and hence a potential contributory factor to myocardial I/R injury (21).

Lipids are essential components of mitochondrial membranes and are required for maintaining the integrity and the proper functioning of mitochondria; however, because of their high content in unsaturated fatty acids, they are particularly vulnerable to peroxidation. It is well known that lipid peroxidation plays a major role in ROS toxicity, resulting in generalized deterioration of mitochondrial membrane structure and function in a number of metabolic disorders, including myocardial I/R injury (6). Among phospholipid species of the inner mitochondrial membrane (IMM), cardiolipin (CL) is of particular importance because of its key role in mitochondrial bioenergetics (88, 91, 93, 106), in mitochondrial dynamics and quality control through fission and fusion (9, 34, 59), in mitophagy to remove damaged mitochondria, as well as in the apoptotic process (90, 111) (Fig. 1). CL is rich in unsaturated fatty acids, which render this compound particularly prone to peroxidation. It is conceivable that oxidative damage to CL would be deleterious to normal mitochondrial function, and this may have important implications in a number of pathophysiological situations, including myocardial I/R injury (22, 71, 93, 113).

In the present review, we focus on the role played by CL alterations in mitochondrial dysfunction during myocardial I/R injury and on the underlying molecular mechanisms. Several pharmacological strategies to prevent or mitigate myocardial I/R injury, directly or indirectly targeting mitochondrial CL, are also examined.

MITOCHONDRIAL FUNCTION IN MYOCARDIAL I/R INJURY

Myocardial I/R causes a number of cardiac injuries including arrhythmias, myocardial stunning, microvascular obstruction, no reflow, and cell death from both necrosis and apoptosis. The sequence of events occurring upon myocardial I/R has been widely investigated and described in several recent reviews (16, 17, 58, 71, 84, 121, 134). Many of the intracellular changes occurring in cardiac I/R can be attributed to the deprivation of oxygen and nutrient supply, which results in a series of abrupt metabolic changes in the myocardium (Fig. 2). The reduced oxygen availability during ischemia alters the OXPHOS process, leading to mitochondrial membrane depolarization, ATP depletion, and inhibition of cardiac contractile function. In the absence of oxygen, cellular metabolism switches to anaerobic glycolysis, which leads to lactate accumulation and cytosolic acidification. The low pH and aberrant ATP-dependent pump/exchanger ion activities result in a net Ca^{2+} accumulation and ROS production inside the cardiomyocyte that may lead to cell death (21, 45, 134).

Reperfusion is an essential intervention to salvage myocardial tissue in acute ischemia and in the early postoperative period subsequent to cardiac surgery. Reperfusion, by restoring myocardial oxygen supply, reestablishes aerobic metabolism, normal cellular homeostasis, cardiomyocyte energization, and salvage of myocardium. However, reperfusion itself comes with a set of problems that exacerbate some of the cytosolic and mitochondrial imbalances, particularly Ca^{2+} and ROS increase, which may contribute, in addition to other factors, to myocardial injury (3, 4, 108). Reperfusion injury may result in increased infarct size, further impairment of myocardial contraction, and the development of reperfusion arrhythmia (33, 45, 58, 71, 84, 134).

Mitochondria synthesize most of the cell's ATP by OXPHOS. Structural and functional integrity of mitochondrial membranes are essential for ATP production and cardiomyocyte function. Myocardial I/R can impair mitochondrial homeostasis, with dramatic consequences on mitochondrial function and cell survival. Impaired mitochondrial energy production, mitochondrial ionic imbalance, and cell stress signaling can lead to mitochondrially mediated cell death (33, 45, 58, 71, 77, 84, 134).

OXIDATIVE STRESS AND MITOCHONDRIAL FUNCTION IN MYOCARDIAL I/R

Mitochondria are considered to be major sources of ROS in a variety of organs, especially in those that are metabolically very active, e.g., the heart. It is known that mitochondria most likely generate ROS during the course of normal OXPHOS process. The mitochondrial electron transport chain (ETC) comprises a series of multisubunit complexes (complexes I–IV) that are coupled to two mobile carriers [coenzyme Q and cytochrome *c* (Cyt *c*)]. Electrons are transferred to molecular oxygen via the ETC, resulting in the reduction of oxygen to water at the level of complex IV. The energy released as electrons move through the ETC is used to transport protons from the mitochondrial matrix to the intermembrane space to

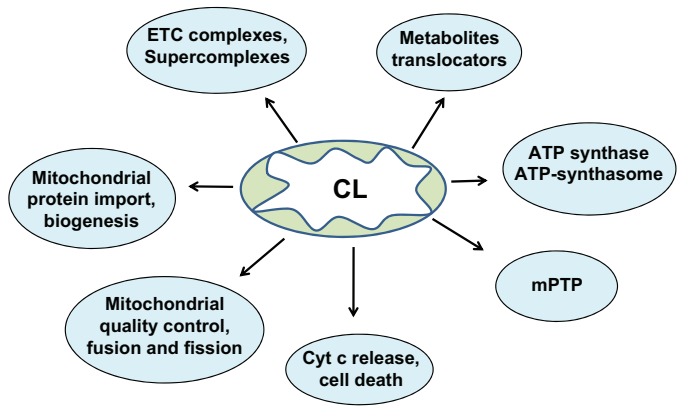

Fig. 1. Proposed roles of cardiolipin (CL) in mitochondrial function. mPTP, mitochondrial permeability transition pore; ETC, electron transport chain; Cyt *c*, cytochrome *c*.

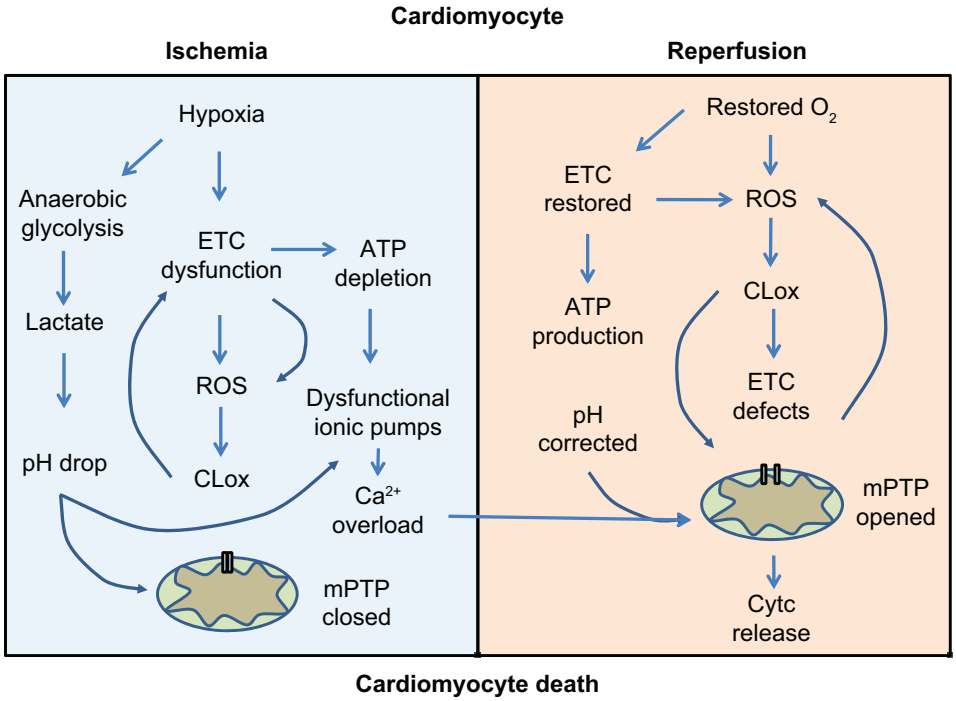

Fig. 2. Schematic illustrating the main proponents of myocardial ischemia-reperfusion injury. During ischemia, hypoxia switches cell metabolism to anaerobic glycolysis, resulting in lactate production and intracellular acidification, a condition that prevents mitochondrial permeability transition pore (mPTP) opening and leads to dysfunctional ion pump activity with subsequent Ca^{2+} overload. The lowered electron transport chain (ETC) activity leads to reactive oxygen species (ROS) production and cardiolipin oxidation, propagating ETC dysfunction and amplifying ROS production. During reperfusion oxidative phosphorylation is restored, leading to ATP production, pH correction, and reactivation of the ion pumps. Because of O_2 burst, ETC activity results in ROS production and cardiolipin oxidation, which further exacerbates ETC dysfunction and ROS production. The restoration of mitochondrial membrane potential drives Ca^{2+} into mitochondria, which, in addition to oxidized cardiolipin (CLox), promote mPTP opening and cytochrome *c* (Cyt *c*) release, leading to cardiomyocyte death.

form an electrochemical gradient that is the major contributor to the IMM potential. Complex V (ATP synthase) uses the stored energy of this proton gradient to synthesize ATP, which is then transported by the adenine nucleotide translocator (ANT) to the intermembrane space in exchange with ADP.

It is estimated that ~0.2–2% of the oxygen taken up by the cell is converted by mitochondria to ROS (19, 21). The rapid movement of electrons through the ETC can result in the leakage of electrons that can form $O_2^{\cdot -}$. The major sites of $O_2^{\cdot -}$ production are at the level of complex I and complex III (86). Complex I releases $O_2^{\cdot -}$ into the matrix, whereas complex III releases $O_2^{\cdot -}$ into both the matrix and intermembrane space. Although complexes I and III are generally considered the major sites of ROS production in I/R, a recent study (23) has suggested that complex II may be an additional site of ROS production. In fact, it has been proposed that superoxide production occurs because of a large accumulation of succinate in the heart during ischemia and its rapid oxidation by reverse electron transport (RET) at the start of reperfusion. This induces a highly reduced state of the ubiquinone binding site on the matrix face of complex I that drives superoxide production (23). The ROS generated by RET may then trigger mitochondrial permeability transition *pore (*mPTP) opening, promoting cardiac injury. This mechanism of superoxide production early in reperfusion has been proposed as a key player in myocardial I/R injury. However, some criticism has been raised in the literature around this proposal (7). In fact, it has been reported that in perfused rat heart subjected to global ischemia increased ROS production during reperfusion primarily occurred after, rather than before, mPTP opening. In addition, succinate accumulation during ischemia is not attenuated by ischemic preconditioning despite powerful cardioprotection (7).

Mitochondria can also produce nitric oxide, which can be converted to various reactive nitrogen species (RNS) such as nitroxyl anion (NO^-) or toxic peroxynitrite ($ONOO^-$), an oxidant that suppresses mitochondrial respiration by modulating the nitration of complexes I and IV (133, 136). Besides interfering with respiratory complexes, these RNS can also trigger free radical-mediated chain reactions that, in turn, damage proteins, lipids, and DNA (21). ROS are also produced in extramitochondrial compartments by enzymatic reactions catalyzed by xanthine oxidase, D-amino acid oxidase, *P*-450 cytochromes, and proline and lysine hydroxylase. ROS-dependent ETC defects can cause a collapse of membrane potential with further generation of ROS, triggering a vicious cycle that ultimately leads to cell death.

ROS AND MITOCHONDRIAL LIPID PEROXIDATION

Phospholipids are essential components of the mitochondrial membranes, where they play multiple structural and functional roles. They modulate proper mitochondrial membrane integrity, permeability, and fluidity, which are required for the optimal functional activities of proteins and enzymes. Mitochondrial membrane phospholipids are rich in polyunsaturated fatty acids that may undergo peroxidation, generating hydroperoxides, endoperoxides, and a broad range of reactive intermediates, among them 4-hydroxy-*trans*-2-nonenal (HNE), one of the most important signaling molecules (126). This compound exhibits a variety of biological activities, including inhibition of protein and DNA synthesis and inactivation of enzymes. HNE is involved in multiple signaling events in a physiological context (132). Recently, in vitro and in vivo studies have provided evidence for the formation of HNE from CL oxidation via cross-chain peroxyl radical addition and decomposition under free radical conditions (132). This finding may have important implications in the apoptotic process and other biological activities of HNE. HNE has also been implicated in the pathogenesis of myocardial I/R (107), and its

removal by mitochondrial isoform aldehyde dehydrogenase 2 is a promising target for pharmaceutical intervention (20). Lipid peroxidation may also indirectly contribute to myocardial I/R injury, because it enhances phospholipid susceptibility to degradation by phospholipases and alters membrane Ca^{2+} permeability and homeostasis (131).

Oxidation of phospholipids is considered one of the major causes of mitochondrial dysfunction in a variety of physiopathological situations, including myocardial I/R injury. In fact, mitochondrial phospholipid oxidation alters the structural and functional organization of the lipid bilayer, changing membrane fluidity and permeability and thereby affecting respiration and the OXPHOS process, maintenance of mitochondrial membrane potential, and mitochondrial Ca^{2+} homeostasis.

CL AND MITOCHONDRIAL FUNCTION

CL is an atypical, dimeric phospholipid located almost exclusively at the level of IMM, where it is also biosynthesized (106). A large body of evidence indicates that this phospholipid plays a key role in mitochondrial bioenergetics (88, 91, 93, 106). In fact, CL interacts with many IMM proteins and enzymes, including, among others, proteins that are components of complexes involved in OXPHOS, such as complexes I, III, IV, V, and Cyt *c*, and in the metabolite translocation across the IMM, such as ANT and phosphate carrier (PiC) (63). Crystallographic studies have shown the presence of a few tightly bound CL molecules in the crystal structures of ETC protein complexes (105) and ANT (63, 135) suggesting that these molecules are integral components of these proteins and are required for their proper folding and optimal functioning. For complexes III and IV an active role of CL in proton translocation has been also suggested (68, 87).

Mitochondrial ETC complexes are organized into supercomplexes or "respirosomes" rather than existing as individual complexes in the IMM (38, 42). Several models of supercomplexes, involving components of the ETC (complexes I, III, and IV), complex V, and ANT, have been proposed (1). In mammalian mitochondria, supercomplexes are comprised of complex I associated with complex III dimers and up to four monomers of complex IV (110). Such supercomplex organization of the ETC provides structural/functional linkages between the individual complexes, thereby facilitating the tight coupling of electron transfer and minimizing the risk of ROS generation. It has been shown that, besides interacting with individual complexes, CL is required for the proper assembly, stabilization, and functioning of the mitochondrial supercomplexes (26, 83). In addition, CL seems to be required for the assembly of ANT, PiC, and ATP synthase into a large supercomplex called ATP synthasome that has been suggested to improve the efficiency of the OXPHOS process (26). CL is linked to the operation of OXPHOS proteins functioning as a proton trap, restricting pumped protons within its phosphate head group domain and supplying protons for the ATP synthase (47).

It is conceivable that CL abnormalities, notably CL oxidation, which may occur under conditions of oxidative stress, can negatively impact the activity and efficiency of ETC protein complexes involved in OXPHOS, thereby affecting mitochondrial bioenergetics (91). Alterations in mitochondrial CL profile may occur as a consequence of loss in CL content due to impaired CL synthase activity, changes in fatty acyl chain composition due to altered CL remodeling, and oxidation due to ROS attack. CL molecules are rich in unsaturated fatty acids, particularly linoleic acid, in heart and liver tissues or docosahexaenoic acid (DHA) and arachidonic acid in brain tissue. This unique acyl composition is not derived from de novo synthesis of CL but rather from a remodeling process in which CL undergoes cycles of deacylation and reacylation (18, 106, 124). In addition, CL remodeling is believed to replace damaged acyl chains under normal conditions. This remodeling process may also generate CL species that are easily susceptible to oxidative damage by ROS under pathological conditions, further exacerbating CL peroxidation and oxidative stress. Thus, altered CL remodeling has been implicated in the etiology of mitochondrial dysfunction associated with a variety of pathophysiological conditions including diabetes, obesity, and cardiovascular diseases, all of which are characterized by increased oxidative stress, CL alterations, and enrichment of DHA content in CL (115, 118). Acyl-CoA:lysocardiolipin acyltransferase (ALCAT)-1 catalyzes the reacylation of lysocardiolipin to CL, a key step in CL remodeling (18, 106, 124). CL remodeling by ALCAT-1 also leads to the synthesis of CL with aberrant acyl composition commonly found in heart disease, including loss of linoleic acid and enrichment of DHA content in CL (115, 118). It has been suggested that CL remodeling by ALCAT-1 may be a common denominator in oxidative stress and mitochondrial dysfunction associated with cardiovascular diseases (73, 74). Consequently, inhibition of ALCAT-1 by chemical reagents may provide a potential treatment for these pathological conditions.

CL molecules are associated with complexes I and III, which are the major mitochondrial source of ROS production. Because of their location in the IMM and because of their high content of polyunsaturated fatty acids, CL molecules are particularly susceptible to oxidation. CL alterations due to oxidation, depletion, and remodeling are emerging as important contributory factors in mitochondrial dysfunction in a variety of metabolic disorders and physiopathological settings, including myocardial I/R, diabetes, aging, and age-related cardiovascular and neurodegenerative disorders (22, 93, 96). Oxidized CL is also emerging as a key player in the modulation of several mitochondrial steps of the apoptotic process (90, 111) and mPTP opening (99). Moreover, it has also been proposed that oxidized CL migrates to the outer mitochondrial membrane (OMM), where it participates in the mitophagic pathway (8).

CL AND MITOCHONDRIAL DYNAMICS

Mitochondria are intimately involved in cell fate during disease; therefore, it is essential to have stringent quality control mechanisms to ensure a healthy mitochondrial network. Quality control mechanisms are largely regulated by mitochondrial dynamics and mitophagy, through processes such as mitochondrial fission and fusion that facilitate the equilibration of mitochondrial components such as DNA, proteins, and metabolites (62). The process of mitophagy is responsible for the degradation and recycling of damaged mitochondria. Recent in vitro and in vivo studies show that CL is involved in several steps of mitochondrial dynamics and

morphology, including fusion and fission processes (9, 59, 62), in mitophagy, and in protein insertion and assembly into the mitochondria (81). The involvement of CL in mitochondrial dynamics can be attributed, most probably, to its unique biochemical and biophysical properties (106). CL is a dimeric phospholipid with a small acid-head group and four long acyl chains, thus rendering it effectively cone shaped. Consequently, CL exhibits complex phase behavior in membrane with a propensity to stabilize negative membrane curvature and to transition from a lamellar, bilayer phase to an inverted hexagonal, nonbilayer configuration. These biophysical properties of CL may be relevant to lipid rearrangement events that occur during mitochondrial fission and fusion processes. Indeed, the involvement of dynamic-related protein-1 (DRP1) in promoting mitochondrial fission is critically dependent on CL interaction (119). It has been proposed that DRP1 and CL function cooperatively in facilitating membrane remodeling and fission during mitochondrial division (119). It is reasonable to hypothesize that CL alterations that occur during heart I/R (41, 72, 92) may alter the interaction between CL and DRP1, thus affecting mitochondrial fission and dynamics and thereby contributing to mitochondrial dysfunction. Impairment of mitochondrial dynamics has been proposed to contribute to the pathogenesis of myocardial I/R (8). It has been shown that during heart reperfusion mitochondria undergo fission, while there is a decrease in mitochondrial fusion (79). Increased mitochondria fission results in an enhanced susceptibility to mPTP opening, which leads to the release of Cyt *c* and other caspase family proteins, resulting in activation of apoptotic cell death at the time of myocardial reperfusion (79). Therefore, mitochondrial fusion and fission proteins may provide novel therapeutic targets for treating several myocardial disorders, such as I/R injury, heart failure, and left ventricular hypertrophy (89).

CL IN MITOPHAGY AND APOPTOSIS

Mitophagy refers to the selective removal of superfluous or damaged mitochondria by autophagosomes (129). This is a critical mechanism in control of mitochondrial quality. In normal functioning mitochondria CL is confined to the matrix-oriented leaflet of the IMM. It has been hypothesized that the migration of CL from the IMM to the OMM in mitochondria may be a message for mitophagy (78). Under low and mild stress conditions, migrated CL on the surface of the OMM combines with light chain 3 (LC3), a member of the Atg8 family in mammals, contributing to the recognition of injured mitochondria and initiating autophagosome formation leading to the development of mitophagy (24). However, oxidation of CL is far greater in response to severe stress than under normal or mild-damage conditions. During apoptotic/high stress conditions, large amounts of ROS are produced, leading to CL oxidation. In addition, CL may form a complex with Cyt *c*, converting it to a peroxidase in the presence of H_2O_2, leading to additional formation of oxidized CL (78). The accumulation of oxidized CL in OMM results in recruitment of Bax and formation of the mPTP, which, in turn, leads to Cyt *c* release from mitochondria to the cytosol, thus triggering the apoptotic process (see below). The different characteristics of CL in apoptosis and mitophagy may result from the various associated oxidation reactions.

ROLE OF CL IN MYOCARDIAL APOPTOSIS IN I/R

Myocardial apoptosis is a cellular death program finely regulated by many regulatory factors. Loss of cardiomyocytes via apoptosis contributes to the decline of ventricular function during myocardial I/R. Cardiac myocytes are differentiated cells that cannot be replaced after death; therefore, their loss compromises the ability of the myocardium to sustain contractile function. Myocardial apoptosis is dominant in the pathogenesis of myocardial I/R and in maintenance of myocardial dysfunction after reperfusion (77, 130). Myocardial ischemia is associated with an enhanced rate of necrosis, whereas reperfusion leads to increased apoptosis (80). It is widely accepted that CL modulates several mitochondrial steps of the apoptotic process leading to cell death (90, 111). The participation of CL in apoptosis is attributed to its association and interaction with a number of cell death-inducing proteins, mainly Cyt *c* (54, 125). CL directly binds to Cyt *c* at the level of the IMM. During apoptotic signaling Cyt *c* is released from the IMM to the cytosol, where it triggers the caspase cascade. Cyt *c* is bound to the outer leaflet of the IMM through both electrostatic and hydrophobic interactions with CL (54). Oxidative damage of CL promotes the detachment of Cyt *c* from the IMM, and this event is considered the initial step in the release of Cyt *c* from mitochondria (104). During the apoptotic process, almost 40% of CL present in the IMM is translocated to the OMM, where it participates in the formation of Cyt *c*-CL complex, which displays peroxidase activity (57). This complex induces CL peroxidation, which, in the presence of Ca^{2+}, may promote mPTP opening (99). This event allows the release of Cyt *c* from mitochondria, thus triggering the execution phase of the apoptotic process. The relevance of Cyt *c*-CL complex peroxidase activity to I/R myocardial injury has recently been shown (5). It has been reported that an increase in mitochondrial ROS production, coupled with electron transfer through the Cyt *c* segment of the ETC, oxidizes Cyt *c* at the Met^{80} residue, followed by CL loss. The content of Cyt *c* methionine oxidation increases after heart I/R (54).

Because of the role of CL in multiple reactions of mitochondrial function and dynamics, it is conceivable that alterations occurring in CL structure, content, and acyl chain composition may have important implications in mitochondrial dysfunction and hence in mitochondrial physiopathology, including myocardial I/R injury.

CL AND MITOCHONDRIAL ETC IN MYOCARDIAL I/R

Mitochondria-mediated ROS generation may contribute to myocardial tissue alterations during I/R. Oxygen free radicals are produced not only during reperfusion and reoxygenation after ischemia or hypoxia but also during ischemia (127) and hypoxia (128). The presence of residual O_2 in cardiomyocyte is a critical element for mitochondrial ROS generation during ischemia, in that electron transfer through the ETC becomes markedly slowed and the respiratory complexes are in a highly reduced state, so that electrons leak to combine with O_2, producing $O_2^{\cdot -}$ radicals. Another possibility is that under a condition of limited O_2 a slower electron transfer through complex IV leads to an increased level of reduced Cyt *c*, thus lowering Cyt *c*-mediated $O_2^{\cdot -}$scavenging capability (117).

CL molecules are an early target for ROS attack because of their high content of linoleic acid and their vicinity to the site

of ROS production in the IMM, although tetralinoleoyl CL in mammalian cells is in a compact configuration, with double bonds relatively shielded from oxidative attack. A decrease in CL level and an increase in CL oxidation have been reported in rat heart mitochondria during ischemia (41, 71, 72, 92). This oxidation/depletion of CL seems to be responsible, in addition to other factors, for the altered activity of respiratory chain complexes I and III (97, 98, 103). The impairment of the activities of these two complexes is one of the earliest ischemia-induced alterations in mitochondrial function. Ischemia-associated CL oxidation may also be implicated in the release of Cyt *c* from mitochondria (13). In fact, as described above, CL oxidation promotes the detachment of Cyt *c* from IMM, which is a requirement for Cyt *c* release from mitochondria. The Cyt *c* release occurs without mPTP opening but is associated with a depletion of Bcl-x_L content that is implicated in the regulation of OMM permeability that leads to Cyt *c* release in apoptosis (13). In addition, oxidized Cyt *c* acts as an effective superoxide scavenger within the mitochondria (117); thus its loss during ischemia may contribute to enhanced mitochondrial oxidative stress and mitochondrial dysfunction.

Ischemic damage to complexes I and III enhances their capacity to generate $O_2^{\cdot -}$, setting the stage for a burst of ROS production upon reperfusion. Moreover, the increased generation of ROS by the ETC complexes may, in turn, damage adjacent complexes, propagating ETC dysfunction and amplifying ROS generation and mitochondrial dysfunction. Studies from our and other laboratories have shown alterations in bioenergetic parameters, such as decreased rate of mitochondrial oxygen consumption, reduced activity of complexes I, III, and IV, and increased basal rate of H_2O_2 production in mitochondria isolated from Langendorff-perfused rat heart subjected to global ischemia (42, 69, 72, 97, 98, 103). These alterations are associated with an oxidation/depletion of CL. Liposomal delivery of exogenous intact CL, but not oxidized CL, to mitochondria isolated from I/R rat heart almost completely restores the activity of these respiratory enzyme complexes to preischemia levels. Other phospholipids, such as phosphatidylcholine and phosphatidylethanolamine, were unable to replace CL in this effect (98). Collectively, these results suggest that the alterations in the respiratory chain complexes in I/R rat heart mitochondria may be due to oxidation/depletion of CL, which is required for the optimal function of these enzyme complexes. This is also supported by the finding that antioxidant compounds, administered before ischemia and during reperfusion, were able to prevent both the oxidation/depletion of CL and the alterations in the activity of these enzyme complexes (2, 41, 101). In addition to CL alterations, other mechanisms have been suggested to be involved in the damage of ETC activity during heart I/R. Complex I undergoes conformational change in response to ischemia, shifting from an active to a deactive form, and complex III iron-sulfur protein is damaged during heart I/R (71).

As mentioned above, mitochondrial ETC complexes assemble in higher-order structures referred to as supercomplexes. CL seems to be required for these supercomplex assemblies and stabilization, in addition to its occurrence as an integral part of individual complexes (26, 83). Because of the oxidation and depletion of mitochondrial CL in myocardial I/R, it is conceivable that these CL alterations may contribute to the destabilization and dysfunction of ETC supercomplexes. Consistent with this, recent studies on mitochondria isolated from I/R rat heart have shown the destabilization of mitochondrial ETC supercomplexes (I, III, and IV) after sustained reperfusion (55). It has also been proposed that upon reperfusion partial recovery of ETC activity without supercomplex reassembly results in altered OXPHOS and enhanced $O_2^{\cdot -}$ production that, in turn, may damage adjacent complexes, propagating ETC dysfunction and amplifying ROS production (46).

CL AND MPTP IN MYOCARDIAL I/R INJURY

Mitochondria are the primary source of ATP, which is needed to drive the contractile cycle and maintain ionic homeostasis in cardiomyocytes. ATP production by OXPHOS requires the permeability barrier of the IMM to be preserved. Under physiological conditions, the IMM is impermeable to all metabolites and ions. Mitochondria contain a latent nonspecific pore within the IMM known as mPTP (10, 11, 48, 50). Opening of this pore induces passive diffusion of any molecules of 1.5 kDa, collapse of membrane potential, uncoupling of OXPHOS, and reversal of the F_0F_1-ATP synthase, causing hydrolysis of ATP that is produced by the glycolytic process. All these events lead to mitochondrial dysfunction and cell death predominantly through necrosis. Despite extensive studies, the exact molecular identity of the mPTP has not been yet elucidated. Several molecules have been proposed as potential candidates in the formation and modulation of the mPTP, including cyclophilin D (Cyp D) in the matrix, ANT and PiC in the IMM, and voltage-dependent anion channels (VDACs) in the OMM (10, 11, 47). Cyh D, a nuclear encoded mitochondrial isoform of cyclophilin, is considered a key component of mPTP, as shown by the inhibition of mPTP opening by cyclosporin A (CsA) (48). ANT is another putative component of the mPTP; however, other studies using knockout mice have shown that this protein carrier is not directly involved in mPTP but rather may have a regulatory role (65). An involvement of PiC in mPTP formation has been also proposed, as shown by the finding that Cyp D binds to this protein in a CsA-sensitive manner (48). More recently, mitochondrial F_0F_1-ATP synthase has been proposed as a molecular component of the mPTP (44, 67). CL molecules have been shown to be associated with ATP synthase and also to be required for its functioning (36). In fact, CL interacts specifically with the rotor of this multisubunit protein complex, either facilitating its rotation or participating directly in the rotation supported by the transmembrane proton motive force (35). The involvement of F_0F_1-ATP synthase in mPTP formation is supported by the finding that Cyp D comigrates with mitochondrial F_0F_1-ATP synthase in blue native gel and the subunit OSCP as a binding site (44). Two potential sites of pore formation in mitochondrial ATP synthase complex have been suggested: the monomer-monomer interface of the dimer and the C ring by itself or in the context of mitochondrial F_0F_1-ATP synthase (12, 44). In mitochondria, F_0F_1-ATP synthase and its associated substrate suppliers, PiC and ANT, can form the ATP synthasome complex (26). It has been proposed that this supercomplex might be involved in the formation/regulation of mPTP (50, 60). The oligomerization of the ATP synthasome seems to be modulated by CL (25), which associates and interacts with all the protein components of this supercomplex, gluing these proteins together. It has also been suggested that Ca^{2+} ions, which are the main inducers of

mPTP opening, bind to annular CL at the interface between PiC, ANT, and F_0F_1-ATP synthase (50). Under conditions promoting mPTP opening, Ca^{2+} binds to CL, triggering conformational changes in the protein components of the ATP synthasome and thus generating the pore. It is reasonable to hypothesize that alterations occurring in the structure, fatty acyl composition, and particularly degree of CL oxidation may disturb the interactions between the protein components of the ATP synthasome, inducing destabilization and conformational changes in this supercomplex, thus favoring, in the presence of Ca^{2+}, mPTP opening (Fig. 3). Additional support for this hypothesis comes from our in vitro study (99) on isolated rat heart mitochondria showing that oxidized CL sensitizes the organelle to Ca^{2+}-induced mPTP opening.

It is well recognized that the mPTP plays a major role in cardiomyocyte cell death during myocardial I/R (50, 51). It has been shown that the mPTP remains closed during ischemia because of the intracellular acidosis caused by lactate accumulation (49). Postischemic reperfusion results in an influx of Ca^{2+} into mitochondria, a burst of ROS production, and a rapid correction of the acidosis, all events that may contribute to the increase in the probability of mPTP opening (Fig. 2).

Ca^{2+} is the most prominent inducers of the mPTP opening (11, 50). In addition to Ca^{2+}, a number of other factors are involved in the activation of mPTP opening, including ROS and oxidized CL (100). Our study (99) on isolated rat heart mitochondria has shown that adding exogenous oxidized CL sensitizes mitochondria to Ca^{2+}-induced mPTP opening. This synergistic effect of Ca^{2+} and oxidized CL on mPTP opening suggests that both these compounds could play a coordinated role in this process by interacting with components of the mPTP, probably ANT, PiC, and F_0F_1-ATP synthase to which CL molecules are tightly associated in the ATP synthasome complex. Given that the mitochondrial levels of Ca^{2+} and oxidized CL increase during I/R, it is reasonable to hypothesize that both of these two factors may contribute to mPTP opening during postischemic heart reperfusion (92, 100). In addition, the induction of mPTP opening by oxidized CL and Ca^{2+} is associated with the release of Cyt *c* from mitochondria, which can lead to cardiomyocyte cell death after myocardial I/R (100). Therefore, preventing CL alterations may provide a novel therapeutic target for treating myocardial I/R injury.

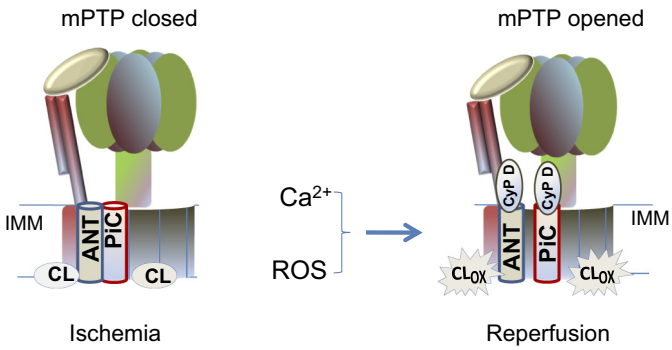

Fig. 3. Proposed mechanism for the involvement of oxidized cardiolipin (CLox) in mitochondrial permeability transition pore (mPTP) opening. IMM, inner mitochondrial membrane; CL, cardiolipin; Cyp D, cyclophilin D; ANT, adenine nucleotide translocator; PiC, phosphate carrier.

Table 1. *Pharmacological agents against mitochondrial dysfunction in myocardial ischemia-reperfusion directly or indirectly preventing cardiolipin alterations*

Compound	References
Melatonin	31, 32, 95, 100, 101
Bendavia peptide	28, 64, 122
Ranolazine	3, 41
Propofol	112

CL-TARGETING THERAPEUTIC AGENTS IN MYOCARDIAL I/R INJURY

Mitochondria play an important role in myocardial I/R injury, as disturbance of mitochondrial bioenergetics and dynamics is a major cause of cardiac dysfunction. Thus mitochondria-targeted pharmacological interventions may provide an effective strategy to prevent or mitigate potential adverse side effects (52). As discussed above, mitochondrial CL alterations are intimately implicated in mitochondrial dysfunction during myocardial I/R injury, by affecting ETC and OXPHOS processes and mitochondrial dynamics and inducing mPTP opening. Several compounds targeting mitochondrial CL directly or indirectly have been shown to provide myocardial protection against I/R injury, and some of them are currently being tested in clinical trials (Table 1). In this section, pharmacological agents that can provide cardioprotection against I/R injury through a CL-mediated effect are examined.

Melatonin, the major secretory product of the pineal gland, is a well-known antioxidant agent that is highly concentrated inside mitochondria, where it exerts an effective antioxidant activity, thus protecting mitochondrial bioenergetic function (95, 123). Melatonin is also validated to maintain a healthy mitochondrial network by modulating mitochondrial biogenesis, dynamics, and mitophagy (27). Melatonin treatment has strong protective effect against mitochondrial bioenergetic alterations in a rat model of myocardial I/R, limiting ROS production and preventing CL alterations and loss of ETC complex I and III activities and of state 3 respiration (95, 101). In addition, melatonin inhibits mPTP opening and the release of Cyt *c* in mitochondria isolated from I/R rat heart as well as in in vitro experiments on isolated rat heart mitochondria (100). The protective effect of melatonin against CL alterations, notably CL oxidation, in myocardial I/R can be reasonably explained by the ability of this compound to prevent the peroxidation of linoleic fatty acids, which are the main constituents of heart CL molecules. In fact, results reported in the literature demonstrate that melatonin and its derivatives display strong in vitro lipid peroxyl radical scavenging properties (82).

The protective effect of melatonin against mitochondrial dysfunction during rat heart I/R is associated with an improvement of postischemic hemodynamic function of the Langendorff heart on reperfusion and reduction in infarct size and necrotic damage (100). This protective effect by melatonin could be explained partly by its ability to preserve CL integrity from ROS attack (102). Collectively, these results emphasize that melatonin-induced mitochondrial adaptive changes are likely of great value for the cardioprotective actions of this indoleamine (32, 75, 94). Because of the lack of side effects, melatonin may represent an effective therapeutic strategy to combat a variety of oxidative stress-induced mitochondria-

related diseases, including myocardial I/R. Moreover, because of the ability of melatonin to protect human cells and reduce infarct size in an animal model of myocardial infarction (MI), the rationale exists to translate these results into the clinical setting (32). Indeed, very recently, it was reported that administration of melatonin in patients with ST segment elevation MI who presented early after symptom onset was associated with a significant reduction in infarct size after primary percutaneous coronary intervention (31). This promising result needs to be validated in larger clinical trials. However, other studies have shown that administration of melatonin did not improve the myocardial salvage index after primary percutaneous coronary intervention in patients with ST elevation during MI compared with placebo (37). The lack of a positive effect could be due to an ineffective dose of melatonin and/or to the timing of administration.

Recent studies have supported the development of a new class of cell-permeant peptides that accumulate inside mitochondria and selectively target CL (122). One of these peptides, MTP-131 (also called Bendavia or elamipretide), was shown to be effective in preserving contractile force after cardiac I/R, significantly reducing infarct size in numerous experimental animal species, including large animals (14, 64, 122). Bendavia, by binding selectively to CL via both electrostatic and hydrophobic interactions, prevents CL from converting Cyt *c* into a peroxidase, interrupting the vicious cycle of ROS-mediated CL oxidation while protecting Cyt *c* electron-carrying function (122). As a result, Bendavia preserves the normal structure of mitochondrial cristae, thus improving respiratory supercomplex assembly and function and the OXPHOS process. Bendavia represents a new class of compounds that can recharge the cellular powerhouse and restore bioenergetics (122). The mechanism of action of this compound appears to be distinct from direct mPTP inhibition, mitochondrial uncoupling, or ROS scavenging activity. In both small- and large-animal experiments, Bendavia has been reported to reduce MI size when administered at the onset of reperfusion and to prevent adverse left ventricular remodeling after MI (28). In addition, Bendavia improves postinfarction cardiac function, restores mitochondrial energy metabolism gene expression, and suppresses cardiac fibrosis in the border zone of the infarcted heart in rats (28, 114). This agent has been also investigated in several phase II clinical trials to evaluate its safety, tolerability, and efficacy on myocardial reperfusion injury in patients. However, in a clinical trial to evaluate myocardial effects of Bendavia for reducing reperfusion injury in a carefully selected population of patients with anterior ST segment elevation MI with acute coronary events, this agent administered before percutaneous coronary intervention was not associated with a decrease in MI size or clinical outcomes (43). Potential reasons for the neutral results of this study may include ineffective dose and timing of administration of this compound and also difficulties of Bendavia to target mitochondria in these patients. Thus, additional studies are needed to confirm the beneficial effect of this compound against myocardial I/R injury.

Mitoquinone (MitoQ), a coenzyme Q analog, selectively accumulates into mitochondria, where it is reduced by the respiratory chain to its active ubiquinol form, which is a particularly effective antioxidant, preventing mitochondrial oxidative damage (85). MitoQ has been shown to protect cardiac function in an animal model of heart I/R by preventing mitochondrial swelling, Cyt *c* release, mitochondrial dysfunction, and cell death (2). It has been suggested that the mechanism of cardioprotection by this agent is likely due to its antioxidant efficacy to prevent phospholipid peroxidation, including CL oxidation. ROS production and mitochondrial dysfunction during cardiac graft reperfusion is a major factor in posttransplant I/R injury. MitoQ has been shown to decrease I/R injury in a murine syngeneic heart transplant model (29).

Cationic derivatives of plastoquinones are another class of antioxidants that selectively accumulate within mitochondria because of their strong negative membrane potential (116). It has been proposed that one of the mechanisms through which these compounds exert their protective action against mitochondrial oxidative damage is the prevention of mitochondrial CL oxidation by ROS attack (39). This protective effect of plastoquinones may underlie their beneficial effects against several physiopathological states, including heart I/R.

Propofol is an anesthetic compound frequently used during cardiac surgery and in postoperative sedation (53). This drug acts as a free radical scavenger and may also inhibit plasma membrane calcium channels. Propofol has been shown to protect the Langendorff-perfused rat heart against reperfusion injury and damage caused by H_2O_2-induced oxidative stress (56). The proposed mechanism for propofol-induced cardioprotection is at the mitochondrial level through the inhibition of mPTP opening and the protection of functional activity of ETC complexes via preservation of CL integrity (112).

Ranolazine is a clinically used drug known to reduce cardiac dysarrhythmias (120) and protect myocardial tissue damage after I/R (41). The cardioprotective effect of ranolazine is associated with preservation of mitochondrial function, more specifically at the level of respiratory chain complex I and its supporting structures such as supercomplexes. CL is bound to complex I, and it is essential for maintaining structural and functional integrity of this complex and for its assembly into supercomplexes (40). During ischemia, mitochondria produce ROS at ETC sites, particularly at the level of complex I, and this contributes to the ischemic damage that, in turn, augments myocyte injury during subsequent reperfusion (41, 70). It has

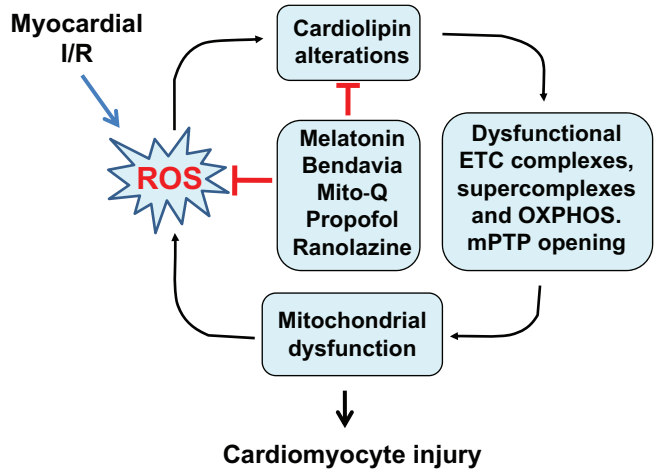

Fig. 4. Involvement of cardiolipin alterations in mitochondrial dysfunction in myocardial ischemia-reperfusion (I/R) and cardioprotective effect by pharmacological agents. ROS, reactive oxygen species; ETC, electron transport chain; OXPHOS, oxidative phosphorylation; mPTP, mitochondrial permeability transition pore. For details see the text.

been reported that ranolazine, when present during ischemia, prevents oxidative damage to CL, thereby maintaining structural and functional integrity of complex I and supercomplexes (41). Preservation of CL integrity and mitochondrial bioenergetics by ranolazine in I/R rat hearts could be ascribed to the ability of this drug to decrease mitochondrial Ca^{2+} overload and mitochondrial ROS production.

CONCLUSIONS AND PERSPECTIVES

I/R injury is an important cause of impaired heart function in the early postoperative period subsequent to cardiac surgery and in acute myocardial ischemia. A large body of evidence supports a crucial role of mitochondrial dysfunction in myocardial I/R injury. Increased ROS generation, defects in ETC activity and the OXPHOS process, mPTP opening, and aberrant mitochondrial dynamics are considered contributory factors to mitochondrial dysfunction in myocardial I/R injury. Accumulating evidence suggests that CL is actively involved in multiple reactions of mitochondrial bioenergetics, dynamics, mitophagy, and apoptosis. Therefore, abnormalities occurring in the CL profile may underlie mitochondrial dysfunction during myocardial I/R as well as in other physiopathological settings. This review highlights the role played by CL alterations in mitochondrial dysfunction associated with myocardial I/R injury. Additional studies are needed that more clearly delineate the molecular mechanisms through which CL alterations affect mitochondrial function in myocardial I/R injury. Pharmacological interventions designed to prevent or reduce CL-related mitochondrial dysfunction would be beneficial in managing myocardial I/R injury. Several compounds directly or indirectly targeting mitochondrial CL have been shown to be effective in protecting mitochondrial function in myocardial I/R (Fig. 4) . The results of larger clinical trials of these interventions are now required to evaluate their therapeutic efficacy and safety and, more widely, the potential of targeting mitochondrial CL for cardioprotection. Mitochondrial dysfunction in the pathogenesis of myocardial I/R injury is a complex process involving different metabolic pathways. Thus, the use of combination therapies targeting mitochondria might have greater cardioprotective efficacy in the clinical setting.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

V.P. and G. Petrosillo prepared figures; G. Paradies, V.P., and G. Petrosillo drafted manuscript; G. Paradies, F.M.R., and G. Petrosillo edited and revised manuscript; G. Paradies and F.M.R. approved final version of manuscript.

REFERENCES

1. **Acin-Perez R, Enriquez JA.** The function of the respiratory supercomplexes: the plasticity model. *Biochim Biophys Acta* 1837: 444–450, 2014. doi:10.1016/j.bbabio.2013.12.009.
2. **Adlam VJ, Harrison JC, Porteous CM, James AM, Smith RA, Murphy MP, Sammut IA.** Targeting an antioxidant to mitochondria decreases cardiac ischemia-reperfusion injury. *FASEB J* 19: 1088–1095, 2005. doi:10.1096/fj.05-3718com.
3. **Aldakkak M, Camara AK, Heisner JS, Yang M, Stowe DF.** Ranolazine reduces Ca^{2+} overload and oxidative stress and improves mitochondrial integrity to protect against ischemia reperfusion injury in isolated hearts. *Pharmacol Res* 64: 381–392, 2011. doi:10.1016/j.phrs.2011.06.018.
4. **Aldakkak M, Stowe DF, Chen Q, Lesnefsky EJ, Camara AK.** Inhibited mitochondrial respiration by amobarbital during cardiac ischaemia improves redox state and reduces matrix Ca^{2+} overload and ROS release. *Cardiovasc Res* 77: 406–415, 2008.
5. **Aluri HS, Simpson DC, Allegood JC, Hu Y, Szczepanek K, Gronert S, Chen Q, Lesnefsky EJ.** Electron flow into cytochrome c coupled with reactive oxygen species from the electron transport chain converts cytochrome c to a cardiolipin peroxidase: role during ischemia-reperfusion. *Biochim Biophys Acta* 1840: 3199–3207, 2014. doi:10.1016/j.bbagen.2014.07.017.
6. **Anderson EJ, Katunga LA, Willis MS.** Mitochondria as a source and target of lipid peroxidation products in healthy and diseased heart. *Clin Exp Pharmacol Physiol* 39: 179–193, 2012. doi:10.1111/j.1440-1681.2011.05641.x.
7. **Andrienko TN, Pasdois P, Pereira GC, Ovens MJ, Halestrap AP.** The role of succinate and ROS in reperfusion injury—a critical appraisal. *J Mol Cell Cardiol* 110: 1–14, 2017. doi:10.1016/j.yjmcc.2017.06.016.
8. **Anzell AR, Maizy R, Przyklenk K, Sanderson TH.** Mitochondrial quality control and disease: insights into ischemia-reperfusion injury. *Mol Neurobiol* 55: 2547–2564, 2018. doi:10.1007/s12035-017-0503-9.
9. **Basu Ball W, Neff JK, Gohil VM.** The role of nonbilayer phospholipids in mitochondrial structure and function. *FEBS Lett* 592: 1273–1290, 2018. doi:10.1002/1873-3468.12887.
10. **Bernardi P, Di Lisa F.** The mitochondrial permeability transition pore: molecular nature and role as a target in cardioprotection. *J Mol Cell Cardiol* 78: 100–106, 2015. doi:10.1016/j.yjmcc.2014.09.023.
11. **Biasutto L, Azzolini M, Szabò I, Zoratti M.** The mitochondrial permeability transition pore in AD 2016: an update. *Biochim Biophys Acta* 1863: 2515–2530, 2016. doi:10.1016/j.bbamcr.2016.02.012.
12. **Bonora M, Bononi A, De Marchi E, Giorgi C, Lebiedzinska M, Marchi S, Patergnani S, Rimessi A, Suski JM, Wojtala A, Wieckowski MR, Kroemer G, Galluzzi L, Pinton P.** Role of the c subunit of the FO ATP synthase in mitochondrial permeability transition. *Cell Cycle* 12: 674–683, 2013. doi:10.4161/cc.23599.
13. **Borutaite V, Brown GC.** Mitochondria in apoptosis of ischemic heart. *FEBS Lett* 541: 1–5, 2003. doi:10.1016/S0014-5793(03)00278-3.
14. **Brown DA, Hale SL, Baines CP, del Rio CL, Hamlin RL, Yueyama Y, Kijtawornrat A, Yeh ST, Frasier CR, Stewart LM, Moukdar F, Shaikh SR, Fisher-Wellman KH, Neufer PD, Kloner RA.** Reduction of early reperfusion injury with the mitochondria-targeting peptide bendavia. *J Cardiovasc Pharmacol Ther* 19: 121–132, 2014. doi:10.1177/1074248413508003.
15. **Camara AK, Aldakkak M, Heisner JS, Rhodes SS, Riess ML, An J, Heinen A, Stowe DF.** ROS scavenging before 27°C ischemia protects hearts and reduces mitochondrial ROS, Ca^{2+} overload, and changes in redox state. *Am J Physiol Cell Physiol* 292: C2021–C2031, 2007. doi:10.1152/ajpcell.00231.2006.
16. **Camara AK, Bienengraeber M, Stowe DF.** Mitochondrial approaches to protect against cardiac ischemia and reperfusion injury. *Front Physiol* 2: 13, 2011. doi:10.3389/fphys.2011.00013.
17. **Camara AK, Lesnefsky EJ, Stowe DF.** Potential therapeutic benefits of strategies directed to mitochondria. *Antioxid Redox Signal* 13: 279–347, 2010. doi:10.1089/ars.2009.2788.
18. **Cao J, Liu Y, Lockwood J, Burn P, Shi Y.** A novel cardiolipin-remodeling pathway revealed by a gene encoding an endoplasmic reticulum-associated acyl-CoA:lysocardiolipin acyltransferase (ALCAT1) in mouse. *J Biol Chem* 279: 31727–31734, 2004. doi:10.1074/jbc.M402930200.
19. **Chance B, Sies H, Boveris A.** Hydroperoxide metabolism in mammalian organs. *Physiol Rev* 59: 527–605, 1979. doi:10.1152/physrev.1979.59.3.527.
20. **Chen CH, Budas GR, Churchill EN, Disatnik MH, Hurley TD, Mochly-Rosen D.** Activation of aldehyde dehydrogenase-2 reduces ischemic damage to the heart. *Science* 321: 1493–1495, 2008. doi:10.1126/science.1158554.
21. **Chen YR, Zweier JL.** Cardiac mitochondria and reactive oxygen species generation. *Circ Res* 114: 524–537, 2014. doi:10.1161/CIRCRESAHA.114.300559.
22. **Chicco AJ, Sparagna GC.** Role of cardiolipin alterations in mitochondrial dysfunction and disease. *Am J Physiol Cell Physiol* 292: C33–C44, 2007. doi:10.1152/ajpcell.00243.2006.
23. **Chouchani ET, Pell VR, Gaude E, Aksentijević D, Sundier SY, Robb EL, Logan A, Nadtochiy SM, Ord EN, Smith AC, Eyassu F, Shirley R, Hu CH, Dare AJ, James AM, Rogatti S, Hartley RC, Eaton S, Costa AS, Brookes PS, Davidson SM, Duchen MR, Saeb-Parsy K, Shattock MJ, Robinson AJ, Work LM, Frezza C, Krieg T, Murphy MP.** Ischaemic accumulation of succinate controls reperfusion injury

through mitochondrial ROS. *Nature* 515: 431–435, 2014. doi:10.1038/nature13909.

24. **Chu CT, Ji J, Dagda RK, Jiang JF, Tyurina YY, Kapralov AA, Tyurin VA, Yanamala N, Shrivastava IH, Mohammadyani D, Wang KZ, Zhu J, Klein-Seetharaman J, Balasubramanian K, Amoscato AA, Borisenko G, Huang Z, Gusdon AM, Cheikhi A, Steer EK, Wang R, Baty C, Watkins S, Bahar I, Bayir H, Kagan VE.** Cardiolipin externalization to the outer mitochondrial membrane acts as an elimination signal for mitophagy in neuronal cells. *Nat Cell Biol* 15: 1197–1205, 2013. doi:10.1038/ncb2837.
25. **Claypool SM, Oktay Y, Boontheung P, Loo JA, Koehler CM.** Cardiolipin defines the interactome of the major ADP/ATP carrier protein of the mitochondrial inner membrane. *J Cell Biol* 182: 937–950, 2008. [Erratum in *J Cell Biol* 197: 1029, 2012.] doi:10.1083/jcb.200801152.
26. **Claypool SM.** Cardiolipin, a critical determinant of mitochondrial carrier protein assembly and function. *Biochim Biophys Acta* 1788: 2059–2068, 2009. doi:10.1016/j.bbamem.2009.04.020.
27. **Coto-Montes A, Boga JA, Rosales-Corral S, Fuentes-Broto L, Tan DX, Reiter RJ.** Role of melatonin in the regulation of autophagy and mitophagy: a review. *Mol Cell Endocrinol* 361: 12–23, 2012. doi:10.1016/j.mce.2012.04.009.
28. **Dai W, Shi J, Gupta RC, Sabbah HN, Hale SL, Kloner RA.** Bendavia, a mitochondria-targeting peptide, improves postinfarction cardiac function, prevents adverse left ventricular remodeling, and restores mitochondria-related gene expression in rats. *J Cardiovasc Pharmacol* 64: 543–553, 2014. doi:10.1097/FJC.0000000000000155.
29. **Dare AJ, Logan A, Prime TA, Rogatti S, Goddard M, Bolton EM, Bradley JA, Pettigrew GJ, Murphy MP, Saeb-Parsy K.** The mitochondria-targeted anti-oxidant MitoQ decreases ischemia-reperfusion injury in a murine syngeneic heart transplant model. *J Heart Lung Transplant* 34: 1471–1480, 2015. doi:10.1016/j.healun.2015.05.007.
30. **Depre C, Taegtmeyer H.** Metabolic aspects of programmed cell survival and cell death in the heart. *Cardiovasc Res* 45: 538–548, 2000. doi:10.1016/S0008-6363(99)00266-7.
31. **Dominguez-Rodriguez A, Abreu-Gonzalez P, de la Torre-Hernandez JM, Consuegra-Sanchez L, Piccolo R, Gonzalez-Gonzalez J, Garcia-Camarero T, Del Mar Garcia-Saiz M, Aldea-Perona A, Reiter RJ, Caballero-Estevez N, de la Rosa A, Virgos-Aller T, Nazco-Casariego J, Laynez-Cerdeña I, Bosa-Ojeda F, Sanchez-Grande A, Yanes-Bowden G, Vargas-Torres M, Lara-Padrón A, Perez-Jorge P, Diaz-Flores L, Lopez J, Lacalzada-Almeida J, Duque A, Bethencourt M, Izquierdo M, Juarez-Prera R, Blanco-Palacios G, Barragan-Acea A, Ferrer-Hita J, Marí-Lopez B, Padilla M, Gonzalez E, Martin-Cabeza M, Mendez-Vargas C, Barrios P, Belleyo-Belkasem C, Leiva M, Betancor I, Miranda J, Giménez Poderós T, Soria-Arcos F, Martinez L; MARIA Investigators.** Usefulness of early treatment with melatonin to reduce infarct size in patients with ST-segment elevation myocardial infarction receiving percutaneous coronary intervention (from the Melatonin Adjunct in the Acute Myocardial Infarction Treated With Angioplasty Trial). *Am J Cardiol* 120: 522–526, 2017. doi:10.1016/j.amjcard.2017.05.018.
32. **Dominguez-Rodriguez A, Abreu-Gonzalez P, Reiter RJ.** Cardioprotection and pharmacological therapies in acute myocardial infarction: challenges in the current era. *World J Cardiol* 6: 100–106, 2014. doi:10.4330/wjc.v6.i3.100.
33. **Dongworth RK, Hall AR, Burke N, Hausenloy DJ.** Targeting mitochondria for cardioprotection: examining the benefit for patients. *Future Cardiol* 10: 255–272, 2014. doi:10.2217/fca.14.6.
34. **Dudek J.** Role of cardiolipin in mitochondrial signaling pathways. *Front Cell Dev Biol* 5: 90, 2017. doi:10.3389/fcell.2017.00090.
35. **Duncan AL, Robinson AJ, Walker JE.** Cardiolipin binds selectively but transiently to conserved lysine residues in the rotor of metazoan ATP synthases. *Proc Natl Acad Sci USA* 113: 8687–8692, 2016. doi:10.1073/pnas.1608396113.
36. **Eble KS, Coleman WB, Hantgan RR, Cunningham CC.** Tightly associated cardiolipin in the bovine heart mitochondrial ATP synthase as analyzed by ^{31}P nuclear magnetic resonance spectroscopy. *J Biol Chem* 265: 19434–19440, 1990.
37. **Ekeloef S, Halladin N, Fonnes S, Jensen SE, Zaremba T, Rosenberg J, Jonsson G, Aarøe J, Gasbjerg LS, Rosenkilde MM, Gögenur I.** Effect of intracoronary and intravenous melatonin on myocardial salvage index in patients with ST-elevation myocardial infarction: a randomized placebo controlled trial. *J Cardiovasc Transl Res* 10: 470–479, 2017. doi:10.1007/s12265-017-9768-7.
38. **Enríquez JA.** Supramolecular organization of respiratory complexes. *Annu Rev Physiol* 78: 533–561, 2016. doi:10.1146/annurev-physiol-021115-105031.
39. **Feniouk BA, Skulachev VP.** Cellular and molecular mechanisms of action of mitochondria-targeted antioxidants. *Curr Aging Sci* 10: 41–48, 2017. doi:10.2174/1874609809666160921113706.
40. **Fiedorczuk K, Letts JA, Degliesposti G, Kaszuba K, Skehel M, Sazanov LA.** Atomic structure of the entire mammalian mitochondrial complex I. *Nature* 538: 406–410, 2016. doi:10.1038/nature19794.
41. **Gadicherla AK, Stowe DF, Antholine WE, Yang M, Camara AK.** Damage to mitochondrial complex I during cardiac ischemia reperfusion injury is reduced indirectly by anti-anginal drug ranolazine. *Biochim Biophys Acta* 1817: 419–429, 2012. doi:10.1016/j.bbabio.2011.11.021.
42. **Genova ML, Lenaz G.** Functional role of mitochondrial respiratory supercomplexes. *Biochim Biophys Acta* 1837: 427–443, 2014. doi:10.1016/j.bbabio.2013.11.002.
43. **Gibson CM, Giugliano RP, Kloner RA, Bode C, Tendera M, Jánosi A, Merkely B, Godlewski J, Halaby R, Korjian S, Daaboul Y, Chakrabarti AK, Spielman K, Neal BJ, Weaver WD.** EMBRACE STEMI study: a Phase 2a trial to evaluate the safety, tolerability, and efficacy of intravenous MTP-131 on reperfusion injury in patients undergoing primary percutaneous coronary intervention. *Eur Heart J* 37: 1296–1303, 2016. doi:10.1093/eurheartj/ehv597.
44. **Giorgio V, von Stockum S, Antoniel M, Fabbro A, Fogolari F, Forte M, Glick GD, Petronilli V, Zoratti M, Szabó I, Lippe G, Bernardi P.** Dimers of mitochondrial ATP synthase form the permeability transition pore. *Proc Natl Acad Sci USA* 110: 5887–5892, 2013. doi:10.1073/pnas.1217823110.
45. **Gottlieb RA.** Cell death pathways in acute ischemia/reperfusion injury. *J Cardiovasc Pharmacol Ther* 16: 233–238, 2011. doi:10.1177/1074248411409581.
46. **Granger DN, Kvietys PR.** Reperfusion injury and reactive oxygen species: the evolution of a concept. *Redox Biol* 6: 524–551, 2015. doi:10.1016/j.redox.2015.08.020.
47. **Haines TH, Dencher NA.** Cardiolipin: a proton trap for oxidative phosphorylation. *FEBS Lett* 528: 35–39, 2002. doi:10.1016/S0014-5793(02)03292-1.
48. **Halestrap AP.** What is the mitochondrial permeability transition pore? *J Mol Cell Cardiol* 46: 821–831, 2009. doi:10.1016/j.yjmcc.2009.02.021.
49. **Halestrap AP, Clarke SJ, Javadov SA.** Mitochondrial permeability transition pore opening during myocardial reperfusion—a target for cardioprotection. *Cardiovasc Res* 61: 372–385, 2004. doi:10.1016/S0008-6363(03)00533-9.
50. **Halestrap AP, Richardson AP.** The mitochondrial permeability transition: a current perspective on its identity and role in ischaemia/reperfusion injury. *J Mol Cell Cardiol* 78: 129–141, 2015. doi:10.1016/j.yjmcc.2014.08.018.
51. **Hausenloy DJ, Yellon DM.** The mitochondrial permeability transition pore: its fundamental role in mediating cell death during ischaemia and reperfusion. *J Mol Cell Cardiol* 35: 339–341, 2003. doi:10.1016/S0022-2828(03)00043-9.
52. **Hausenloy DJ, Barrabes JA, Bøtker HE, Davidson SM, Di Lisa F, Downey J, Engstrom T, Ferdinandy P, Carbrera-Fuentes HA, Heusch G, Ibanez B, Iliodromitis EK, Inserte J, Jennings R, Kalia N, Kharbanda R, Lecour S, Marber M, Miura T, Ovize M, Perez-Pinzon MA, Piper HM, Przyklenk K, Schmidt MR, Redington A, Ruiz-Meana M, Vilahur G, Vinten-Johansen J, Yellon DM, Garcia-Dorado D.** Ischaemic conditioning and targeting reperfusion injury: a 30 year voyage of discovery. *Basic Res Cardiol* 111: 70, 2016. doi:10.1007/s00395-016-0588-8.
53. **Heusch G.** Remote ischemic conditioning in cardiovascular surgery. *J Cardiovasc Pharmacol Ther* 22: 297–301, 2017. doi:10.1177/1074248416687874.
54. **Hüttemann M, Pecina P, Rainbolt M, Sanderson TH, Kagan VE, Samavati L, Doan JW, Lee I.** The multiple functions of cytochrome c and their regulation in life and death decisions of the mammalian cell: from respiration to apoptosis. *Mitochondrion* 11: 369–381, 2011. doi:10.1016/j.mito.2011.01.010.
55. **Jang S, Lewis TS, Powers C, Khuchua Z, Baines CP, Wipf P, Javadov S.** Elucidating mitochondrial electron transport chain supercomplexes in the heart during ischemia-reperfusion. *Antioxid Redox Signal* 27: 57–69, 2017. doi:10.1089/ars.2016.6635.
56. **Javadov SA, Lim KH, Kerr PM, Suleiman MS, Angelini GD, Halestrap AP.** Protection of hearts from reperfusion injury by propofol is

associated with inhibition of the mitochondrial permeability transition. *Cardiovasc Res* 45: 360–369, 2000. doi:10.1016/S0008-6363(99)00365-X.

57. **Kagan VE, Tyurin VA, Jiang J, Tyurina YY, Ritov VB, Amoscato AA, Osipov AN, Belikova NA, Kapralov AA, Kini V, Vlasova II, Zhao Q, Zou M, Di P, Svistunenko DA, Kurnikov IV, Borisenko GG.** Cytochrome c acts as a cardiolipin oxygenase required for release of proapoptotic factors. *Nat Chem Biol* 1: 223–232, 2005. doi:10.1038/nchembio727.
58. **Kalogeris T, Baines CP, Krenz M, Korthuis RJ.** Cell biology of ischemia/reperfusion injury. *Int Rev Cell Mol Biol* 298: 229–317, 2012. doi:10.1016/B978-0-12-394309-5.00006-7.
59. **Kameoka S, Adachi Y, Okamoto K, Iijima M, Sesaki H.** Phosphatidic acid and cardiolipin coordinate mitochondrial dynamics. *Trends Cell Biol*, 28: 67–76, 2018. doi:10.1016/j.tcb.2017.08.011.
60. **Karch J, Molkentin JD.** Identifying the components of the elusive mitochondrial permeability transition pore. *Proc Natl Acad Sci USA* 111: 10396–10397, 2014. doi:10.1073/pnas.1410104111.
61. **Kevin LG, Camara AK, Riess ML, Novalija E, Stowe DF.** Ischemic preconditioning alters real-time measure of O_2 radicals in intact hearts with ischemia and reperfusion. *Am J Physiol Heart Circ Physiol* 284: H566–H574, 2003. doi:10.1152/ajpheart.00711.2002.
62. **Kiriyama Y, Nochi H.** Intra- and intercellular quality control mechanisms of mitochondria. *Cells* 7: E1, 2017. doi:10.3390/cells7010001.
63. **Klingenberg M.** Cardiolipin and mitochondrial carriers. *Biochim Biophys Acta* 1788: 2048–2058, 2009. doi:10.1016/j.bbamem.2009.06.007.
64. **Kloner RA, Hale SL, Dai W, Gorman RC, Shuto T, Koomalsingh KJ, Gorman JH 3rd, Sloan RC, Frasier CR, Watson CA, Bostian PA, Kypson AP, Brown DA.** Reduction of ischemia/reperfusion injury with bendavia, a mitochondria-targeting cytoprotective peptide. *J Am Heart Assoc* 1: e001644, 2012. doi:10.1161/JAHA.112.001644.
65. **Kokoszka JE, Waymire KG, Levy SE, Sligh JE, Cai J, Jones DP, MacGregor GR, Wallace DC.** The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. *Nature* 427: 461–465, 2004. [Erratum in *Nature* 430: 984, 2004.] doi:10.1038/nature02229.
66. **Kurian GA, Rajagopal R, Vedantham S, Rajesh M.** The role of oxidative stress in myocardial ischemia and reperfusion injury and remodeling: revisited. *Oxid Med Cell Longev* 2016: 1656450, 2016. doi:10.1155/2016/1656450.
67. **Laage S, Tao Y, McDermott AE.** Cardiolipin interaction with subunit c of ATP synthase: solid-state NMR characterization. *Biochim Biophys Acta* 1848: 260–265, 2015. doi:10.1016/j.bbamem.2014.08.021.
68. **Lange C, Nett JH, Trumpower BL, Hunte C.** Specific roles of protein-phospholipid interactions in the yeast cytochrome bc1 complex structure. *EMBO J* 20: 6591–6600, 2001. doi:10.1093/emboj/20.23.6591.
69. **Lee HL, Chen CL, Yeh ST, Zweier JL, Chen YR.** Biphasic modulation of the mitochondrial electron transport chain in myocardial ischemia and reperfusion. *Am J Physiol Heart Circ Physiol* 302: H1410–H1422, 2012. doi:10.1152/ajpheart.00731.2011.
70. **Lesnefsky EJ, Chen Q, Moghaddas S, Hassan MO, Tandler B, Hoppel CL.** Blockade of electron transport during ischemia protects cardiac mitochondria. *J Biol Chem* 279: 47961–47967, 2004. doi:10.1074/jbc.M409720200.
71. **Lesnefsky EJ, Chen Q, Tandler B, Hoppel CL.** Mitochondrial dysfunction and myocardial ischemia-reperfusion: implications for novel therapies. *Annu Rev Pharmacol Toxicol* 57: 535–565, 2017. doi:10.1146/annurev-pharmtox-010715-103335.
72. **Lesnefsky EJ, Minkler P, Hoppel CL.** Enhanced modification of cardiolipin during ischemia in the aged heart. *J Mol Cell Cardiol* 46: 1008–1015, 2009. doi:10.1016/j.yjmcc.2009.03.007.
73. **Li J, Romestaing C, Han X, Li Y, Hao X, Wu Y, Sun C, Liu X, Jefferson LS, Xiong J, Lanoue KF, Chang Z, Lynch CJ, Wang H, Shi Y.** Cardiolipin remodeling by ALCAT1 links oxidative stress and mitochondrial dysfunction to obesity. *Cell Metab* 12: 154–165, 2010. doi:10.1016/j.cmet.2010.07.003.
74. **Liu X, Ye B, Miller S, Yuan H, Zhang H, Tian L, Nie J, Imae R, Arai H, Li Y, Cheng Z, Shi Y.** Ablation of ALCAT1 mitigates hypertrophic cardiomyopathy through effects on oxidative stress and mitophagy. *Mol Cell Biol* 32: 4493–4504, 2012. doi:10.1128/MCB.01092-12.
75. **Ma Z, Xin Z, Di W, Yan X, Li X, Reiter RJ, Yang Y.** Melatonin and mitochondrial function during ischemia/reperfusion injury. *Cell Mol Life Sci* 74: 3989–3998, 2017. doi:10.1007/s00018-017-2618-6. [Erratum in *Cell Mol Life Sci* 75: 2681, 2018.]
76. **Maack C, O'Rourke B.** Excitation-contraction coupling and mitochondrial energetics. *Basic Res Cardiol* 102: 369–392, 2007. doi:10.1007/s00395-007-0666-z.
77. **Machado NG, Alves MG, Carvalho RA, Oliveira PJ.** Mitochondrial involvement in cardiac apoptosis during ischemia and reperfusion: can we close the box? *Cardiovasc Toxicol* 9: 211–227, 2009. doi:10.1007/s12012-009-9055-1.
78. **Maguire JJ, Tyurina YY, Mohammadyani D, Kapralov AA, Anthonymuthu TS, Qu F, Amoscato AA, Sparvero LJ, Tyurin VA, Planas-Iglesias J, He RR, Klein-Seetharaman J, Bayır H, Kagan VE.** Known unknowns of cardiolipin signaling: the best is yet to come. *Biochim Biophys Acta* 1862: 8–24, 2017. doi:10.1016/j.bbalip.2016.08.001.
79. **Maneechote C, Palee S, Chattipakorn SC, Chattipakorn N.** Roles of mitochondrial dynamics modulators in cardiac ischaemia/reperfusion injury. *J Cell Mol Med* 21: 2643–2653, 2017. doi:10.1111/jcmm.13330.
80. **Mani K.** Programmed cell death in cardiac myocytes: strategies to maximize post-ischemic salvage. *Heart Fail Rev* 13: 193–209, 2008. doi:10.1007/s10741-007-9073-7.
81. **Marom M, Safonov R, Amram S, Avneon Y, Nachliel E, Gutman M, Zohary K, Azem A, Tsfadia Y.** Interaction of the Tim44 C-terminal domain with negatively charged phospholipids. *Biochemistry* 48: 11185–11195, 2009. doi:10.1021/bi900998v.
82. **Mekhloufi J, Bonnefont-Rousselot D, Yous S, Lesieur D, Couturier M, Thérond P, Legrand A, Jore D, Gardès-Albert M.** Antioxidant activity of melatonin and a pinoline derivative on linoleate model system. *J Pineal Res* 39: 27–33, 2005. doi:10.1111/j.1600-079X.2005.00208.x.
83. **Mileykovskaya E, Dowhan W.** Cardiolipin-dependent formation of mitochondrial respiratory supercomplexes. *Chem Phys Lipids* 179: 42–48, 2014. doi:10.1016/j.chemphyslip.2013.10.012.
84. **Murphy E, Steenbergen C.** Mechanisms underlying acute protection from cardiac ischemia-reperfusion injury. *Physiol Rev* 88: 581–609, 2008. doi:10.1152/physrev.00024.2007.
85. **Murphy MP, Smith RA.** Targeting antioxidants to mitochondria by conjugation to lipophilic cations. *Annu Rev Pharmacol Toxicol* 47: 629–656, 2007. doi:10.1146/annurev.pharmtox.47.120505.105110.
86. **Murphy MP.** How mitochondria produce reactive oxygen species. *Biochem J* 417: 1–13, 2009. doi:10.1042/BJ20081386.
87. **Musatov A, Robinson NC.** Bound cardiolipin is essential for cytochrome c oxidase proton translocation. *Biochimie* 105: 159–164, 2014. doi:10.1016/j.biochi.2014.07.005.
88. **Musatov A, Sedlák E.** Role of cardiolipin in stability of integral membrane proteins. *Biochimie* 142: 102–111, 2017. doi:10.1016/j.biochi.2017.08.013.
89. **Ong SB, Samangouei P, Kalkhoran SB, Hausenloy DJ.** The mitochondrial permeability transition pore and its role in myocardial ischemia reperfusion injury. *J Mol Cell Cardiol* 78: 23–34, 2015. doi:10.1016/j.yjmcc.2014.11.005.
90. **Ott M, Zhivotovsky B, Orrenius S.** Role of cardiolipin in cytochrome c release from mitochondria. *Cell Death Differ* 14: 1243–1247, 2007. doi:10.1038/sj.cdd.4402135.
91. **Paradies G, Paradies V, De Benedictis V, Ruggiero FM, Petrosillo G.** Functional role of cardiolipin in mitochondrial bioenergetics. *Biochim Biophys Acta* 1837: 408–417, 2014. doi:10.1016/j.bbabio.2013.10.006.
92. **Paradies G, Paradies V, Ruggiero FM, Petrosillo G.** Cardiolipin alterations and mitochondrial dysfunction in heart ischemia/reperfusion. *Clin Lipidol* 10: 415–429, 2015. doi:10.2217/clp.15.31.
93. **Paradies G, Paradies V, Ruggiero FM, Petrosillo G.** Cardiolipin and mitochondrial function in health and disease. *Antioxid Redox Signal* 20: 1925–1953, 2014. doi:10.1089/ars.2013.5280.
94. **Paradies G, Paradies V, Ruggiero FM, Petrosillo G.** Protective role of melatonin in mitochondrial dysfunction and related disorders. *Arch Toxicol* 89: 923–939, 2015. doi:10.1007/s00204-015-1475-z.
95. **Paradies G, Petrosillo G, Paradies V, Reiter RJ, Ruggiero FM.** Melatonin, cardiolipin and mitochondrial bioenergetics in health and disease. *J Pineal Res* 48: 297–310, 2010. doi:10.1111/j.1600-079X.2010.00759.x.
96. **Paradies G, Petrosillo G, Paradies V, Ruggiero FM.** Oxidative stress, mitochondrial bioenergetics, and cardiolipin in aging. *Free Radic Biol Med* 48: 1286–1295, 2010. doi:10.1016/j.freeradbiomed.2010.02.020.
97. **Paradies G, Petrosillo G, Pistolese M, Di Venosa N, Federici A, Ruggiero FM.** Decrease in mitochondrial complex I activity in ischemic/reperfused rat heart: involvement of reactive oxygen species and cardiolipin. *Circ Res* 94: 53–59, 2004. doi:10.1161/01.RES.0000109416.56608.64.

98. **Paradies G, Petrosillo G, Pistolese M, Di Venosa N, Serena D, Ruggiero FM.** Lipid peroxidation and alterations to oxidative metabolism in mitochondria isolated from rat heart subjected to ischemia and reperfusion. *Free Radic Biol Med* 27: 42–50, 1999. doi:10.1016/S0891-5849(99)00032-5.
99. **Petrosillo G, Casanova G, Matera M, Ruggiero FM, Paradies G.** Interaction of peroxidized cardiolipin with rat-heart mitochondrial membranes: induction of permeability transition and cytochrome c release. *FEBS Lett* 580: 6311–6316, 2006. doi:10.1016/j.febslet.2006.10.036.
100. **Petrosillo G, Colantuono G, Moro N, Ruggiero FM, Tiravanti E, Di Venosa N, Fiore T, Paradies G.** Melatonin protects against heart ischemia-reperfusion injury by inhibiting mitochondrial permeability transition pore opening. *Am J Physiol Heart Circ Physiol* 297: H1487–H1493, 2009. doi:10.1152/ajpheart.00163.2009.
101. **Petrosillo G, Di Venosa N, Pistolese M, Casanova G, Tiravanti E, Colantuono G, Federici A, Paradies G, Ruggiero FM.** Protective effect of melatonin against mitochondrial dysfunction associated with cardiac ischemia-reperfusion: role of cardiolipin. *FASEB J* 20: 269–276, 2006. doi:10.1096/fj.05-4692com.
102. **Petrosillo G, Moro N, Ruggiero FM, Paradies G.** Melatonin inhibits cardiolipin peroxidation in mitochondria and prevents the mitochondrial permeability transition and cytochrome c release. *Free Radic Biol Med* 47: 969–974, 2009. doi:10.1016/j.freeradbiomed.2009.06.032.
103. **Petrosillo G, Ruggiero FM, Di Venosa N, Paradies G.** Decreased complex III activity in mitochondria isolated from rat heart subjected to ischemia and reperfusion: role of reactive oxygen species and cardiolipin. *FASEB J* 17: 714–716, 2003. doi:10.1096/fj.02-0729fje.
104. **Petrosillo G, Ruggiero FM, Pistolese M, Paradies G.** Reactive oxygen species generated from the mitochondrial electron transport chain induce cytochrome c dissociation from beef-heart submitochondrial particles via cardiolipin peroxidation. Possible role in the apoptosis. *FEBS Lett* 509: 435–438, 2001. doi:10.1016/S0014-5793(01)03206-9.
105. **Pöyry S, Cramariuc O, Postila PA, Kaszuba K, Sarewicz M, Osyczka A, Vattulainen I, Róg T.** Atomistic simulations indicate cardiolipin to have an integral role in the structure of the cytochrome bc1 complex. *Biochim Biophys Acta* 1827: 769–778, 2013. doi:10.1016/j.bbabio.2013.03.005.
106. **Ren M, Phoon CK, Schlame M.** Metabolism and function of mitochondrial cardiolipin. *Prog Lipid Res* 55: 1–16, 2014. doi:10.1016/j.plipres.2014.04.001.
107. **Renner A, Sagstetter MR, Harms H, Lange V, Götz ME, Elert O.** Formation of 4-hydroxy-2-nonenal protein adducts in the ischemic rat heart after transplantation. *J Heart Lung Transplant* 24: 730–736, 2005. doi:10.1016/j.healun.2004.02.021.
108. **Riess ML, Camara AK, Kevin LG, An J, Stowe DF.** Reduced reactive O_2 species formation and preserved mitochondrial NADH and $[Ca^{2+}]$ levels during short-term 17°C ischemia in intact hearts. *Cardiovasc Res* 61: 580–590, 2004. doi:10.1016/j.cardiores.2003.09.016.
109. **Rodríguez-Sinovas A, Abdallah Y, Piper HM, Garcia-Dorado D.** Reperfusion injury as a therapeutic challenge in patients with acute myocardial infarction. *Heart Fail Rev* 12: 207–216, 2007. doi:10.1007/s10741-007-9039-9.
110. **Schägger H, Pfeiffer K.** Supercomplexes in the respiratory chains of yeast and mammalian mitochondria. *EMBO J* 19: 1777–1783, 2000. doi:10.1093/emboj/19.8.1777.
111. **Schug ZT, Gottlieb E.** Cardiolipin acts as a mitochondrial signalling platform to launch apoptosis. *Biochim Biophys Acta* 1788: 2022–2031, 2009. doi:10.1016/j.bbamem.2009.05.004.
112. **Shao H, Li J, Zhou Y, Ge Z, Fan J, Shao Z, Zeng Y.** Dose-dependent protective effect of propofol against mitochondrial dysfunction in ischaemic/reperfused rat heart: role of cardiolipin. *Br J Pharmacol* 153: 1641–1649, 2008. doi:10.1038/bjp.2008.45.
113. **Shen Z, Ye C, McCain K, Greenberg ML.** The role of cardiolipin in cardiovascular health. *BioMed Res Int* 2015: 891707, 2015. doi:10.1155/2015/891707.
114. **Shi J, Dai W, Hale SL, Brown DA, Wang M, Han X, Kloner RA.** Bendavia restores mitochondrial energy metabolism gene expression and suppresses cardiac fibrosis in the border zone of the infarcted heart. *Life Sci* 141: 170–178, 2015. doi:10.1016/j.lfs.2015.09.022.
115. **Shi Y.** Emerging roles of cardiolipin remodeling in mitochondrial dysfunction associated with diabetes, obesity, and cardiovascular diseases. *J Biomed Res* 24: 6–15, 2010. doi:10.1016/S1674-8301(10)60003-6.
116. **Skulachev VP, Antonenko YN, Cherepanov DA, Chernyak BV, Izyumov DS, Khailova LS, Klishin SS, Korshunova GA, Lyamzaev KG, Pletjushkina OY, Roginsky VA, Rokitskaya TI, Severin FF, Severina II, Simonyan RA, Skulachev MV, Sumbatyan NV, Sukhanova EI, Tashlitsky VN, Trendeleva TA, Vyssokikh MY, Zvyagilskaya RA.** Prevention of cardiolipin oxidation and fatty acid cycling as two antioxidant mechanisms of cationic derivatives of plastoquinone (SkQs). *Biochim Biophys Acta* 1797: 878–889, 2010. doi:10.1016/j.bbabio.2010.03.015.
117. **Skulachev VP.** Cytochrome c in the apoptotic and antioxidant cascades. *FEBS Lett* 423: 275–280, 1998. doi:10.1016/S0014-5793(98)00061-1.
118. **Sparagna GC, Lesnefsky EJ.** Cardiolipin remodeling in the heart. *J Cardiovasc Pharmacol* 53: 290–301, 2009. doi:10.1097/FJC.0b013e31819b5461.
119. **Stepanyants N, Macdonald PJ, Francy CA, Mears JA, Qi X, Ramachandran R.** Cardiolipin's propensity for phase transition and its reorganization by dynamin-related protein 1 form a basis for mitochondrial membrane fission. *Mol Biol Cell* 26: 3104–3116, 2015. doi:10.1091/mbc.e15-06-0330.
120. **Stone PH, Chaitman BR, Stocke K, Sano J, DeVault A, Koch GG.** The anti-ischemic mechanism of action of ranolazine in stable ischemic heart disease. *J Am Coll Cardiol* 56: 934–942, 2010. doi:10.1016/j.jacc.2010.04.042.
121. **Stowe DF, Camara AK.** Mitochondrial reactive oxygen species production in excitable cells: modulators of mitochondrial and cell function. *Antioxid Redox Signal* 11: 1373–1414, 2009. doi:10.1089/ars.2008.2331.
122. **Szeto HH.** First-in-class cardiolipin-protective compound as a therapeutic agent to restore mitochondrial bioenergetics. *Br J Pharmacol* 171: 2029–2050, 2014. doi:10.1111/bph.12461.
123. **Tan DX, Manchester LC, Qin L, Reiter RJ.** Melatonin: a mitochondrial targeting molecule involving mitochondrial protection and dynamics. *Int J Mol Sci* 17: E2124, 2016. doi:10.3390/ijms17122124.
124. **Taylor WA, Hatch GM.** Identification of the human mitochondrial linoleoyl-coenzyme A monolysocardiolipin acyltransferase (MLCL AT-1). *J Biol Chem* 284: 30360–30371, 2009. doi:10.1074/jbc.M109.048322.
125. **Tuominen EK, Wallace CJ, Kinnunen PK.** Phospholipid-cytochrome c interaction: evidence for the extended lipid anchorage. *J Biol Chem* 277: 8822–8826, 2002. doi:10.1074/jbc.M200056200.
126. **Uchida K.** 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. *Prog Lipid Res* 42: 318–343, 2003. doi:10.1016/S0163-7827(03)00014-6.
127. **Vanden Hoek TL, Becker LB, Shao Z, Li C, Schumacker PT.** Reactive oxygen species released from mitochondria during brief hypoxia induce preconditioning in cardiomyocytes. *J Biol Chem* 273: 18092–18098, 1998. doi:10.1074/jbc.273.29.18092.
128. **Vanden Hoek TL, Li C, Shao Z, Schumacker PT, Becker LB.** Significant levels of oxidants are generated by isolated cardiomyocytes during ischemia prior to reperfusion. *J Mol Cell Cardiol* 29: 2571–2583, 1997. doi:10.1006/jmcc.1997.0497.
129. **Wang K, Klionsky DJ.** Mitochondria removal by autophagy. *Autophagy* 7: 297–300, 2011. doi:10.4161/auto.7.3.14502.
130. **Webster KA.** Mitochondrial membrane permeabilization and cell death during myocardial infarction: roles of calcium and reactive oxygen species. *Future Cardiol* 8: 863–884, 2012. doi:10.2217/fca.12.58.
131. **Weglicki WB, Dickens BF, Mak IT.** Enhanced lysosomal phospholipid degradation and lysophospholipid production due to free radicals. *Biochem Biophys Res Commun* 124: 229–235, 1984. doi:10.1016/0006-291X(84)90941-0.
132. **Xiao M, Zhong H, Xia L, Tao Y, Yin H.** Pathophysiology of mitochondrial lipid oxidation: role of 4-hydroxynonenal (4-HNE) and other bioactive lipids in mitochondria. *Free Radic Biol Med* 111: 316–327, 2017. doi:10.1016/j.freeradbiomed.2017.04.363.
133. **Yang M, Camara AK, Wakim BT, Zhou Y, Gadicherla AK, Kwok WM, Stowe DF.** Tyrosine nitration of voltage-dependent anion channels in cardiac ischemia-reperfusion: reduction by peroxynitrite scavenging. *Biochim Biophys Acta* 1817: 2049–2059, 2012. doi:10.1016/j.bbabio.2012.06.004.
134. **Yellon DM, Hausenloy DJ.** Myocardial reperfusion injury. *N Engl J Med* 357: 1121–1135, 2007. doi:10.1056/NEJMra071667.
135. **Zhao L, Wang S, Run C, OuYang B, Chou JJ.** Specific lipid binding of membrane proteins in detergent micelles characterized by NMR and molecular dynamics. *Biochemistry* 55: 5317–5320, 2016. doi:10.1021/acs.biochem.6b00836.
136. **Zhou T, Chuang CC, Zuo L.** Molecular characterization of reactive oxygen species in myocardial ischemia-reperfusion injury. *BioMed Res Int* 2015: 864946, 2015. doi:10.1155/2015/864946.