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Mitochondrial Dysfunction and Myocardial Ischemia-Reperfusion: Implications for Novel Therapies

Edward J. Lesnefsky,^{1,2} Qun Chen,¹ Bernard Tandler,³ and Charles L. Hoppel^{4,5,6}

¹Department of Medicine, Division of Cardiology, Pauley Heart Center, Virginia Commonwealth University, Richmond, Virginia 23298; email: edward.lesnefsky@vcuhealth.org, qun.chen@vcuhealth.org

²Medical Service, McGuire Veterans Affairs Medical Center, Richmond, Virginia 23249; email: edward.lesnefsky@va.gov

³Department of Biological Sciences, Case Western Reserve University School of Dental Medicine, Cleveland, Ohio 44106; email: bernard.tandler@case.edu

⁴Department of Pharmacology, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106; email: charles.hoppel@case.edu

⁵Department of Medicine, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106

⁶Center for Mitochondrial Disease, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106

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Abstract

Mitochondria have emerged as key participants in and regulators of myocardial injury during ischemia and reperfusion. This review examines the sites of damage to cardiac mitochondria during ischemia and focuses on the impact of these defects. The concept that mitochondrial damage during ischemia leads to cardiac injury during reperfusion is addressed. The mechanisms that translate ischemic mitochondrial injury into cellular damage, during both ischemia and early reperfusion, are examined. Next, we discuss strategies that modulate and counteract these mechanisms of mitochondrial-driven injury. The new concept that mitochondria are not merely stochastic sites of oxidative and calcium-mediated injury but that they activate cellular responses of mitochondrial remodeling and cellular reactions that modulate the balance between cell death and recovery is reviewed, and the therapeutic implications of this concept are discussed.

ETC: electron transport chain

ROS: reactive oxygen species

MPTP: mitochondrial permeability transition pore

MOMP: mitochondrial outer membrane permeabilization

INTRODUCTION

Mitochondria are critical targets and the root of tissue injury, particularly cardiac injury (the focus of this review), during ischemia and subsequent reperfusion, sometimes termed I/R (1, 2). Occlusion of a coronary artery results in severe, abrupt limitation of blood flow to the myocardium. Sudden occlusion occurring in clinical settings is most often due to thrombosis of a coronary artery, underlain by an atherosclerotic plaque. A severe reduction in blood flow, usually to 10% or less of normal resting perfusion, sets in motion deleterious cellular processes, especially damage to mitochondria, which result in eventual cardiomyocyte death. The imbalance of energy supply and demand is greatest in the endocardium, leading to the onset of cell death in this innermost region of the ventricular wall, starting approximately 20 min after flow cessation. Cell death then proceeds in a transmural manner across the ventricular wall from the endocardium (next to the ventricular cavity) to the epicardium (outermost layer) over approximately 6 h (3, 4). At any given time following coronary occlusion, there are regions of dead myocardium, reversibly injured myocardium, and hypoperfused but viable myocardium. The aim of restoration of blood flow by the clinical use of emergency cardiac catheterization and coronary stent deployment or by administration of thrombolytic therapy is to restore arterial patency and flow—reperfusion—to limit the progression of ischemic necrosis. The goal is to apply this critical therapy within 90 min of clinical presentation, ideally within 6 h from the onset of artery thrombosis. Timely reperfusion results in improved left ventricular postinfarction contractile function and improved patient survival. Although resumption of flow limits damage from ischemia, salvage of jeopardized cardiac myocytes that have undergone cell injury during the ischemic period, but not yet sustained ischemic cell death, is frustratingly suboptimal (3). In fact, additional myocyte death occurs during the reperfusion period (2), not from ischemic necrosis, but from altered metabolism of restored oxygen and substrates by ischemia-damaged mitochondria. In experimental models, 25–40% of eventual cardiac cell death is related to reperfusion-induced death. Damage to cardiac mitochondria that leads to deranged mitochondrial metabolism during the early reperfusion period is a key contributing mechanism to this second occurrence in the progression of myocardial cell death. Nonetheless, despite timely and successful reperfusion, many patients with acute coronary thrombosis leading to acute myocardial infarction sustain substantial damage to cardiac tissue.

In the setting of severe myocardial ischemia, damage to the electron transport chain (ETC) occurs mainly during the ischemic period (1). Reperfusion of myocardium that contains mitochondria damaged by the preceding period of severe ischemia leads to early mitochondrial-driven injury with excessive production of reactive oxygen species (ROS) and dysregulation of calcium, leading to abnormal permeation, along with swelling and disruption of mitochondria by mitochondrial permeability transition pore (MPTP) (5, 6). The mechanisms of early reperfusion oxidative and calcium-mediated injury have been discussed extensively by others and are summarized only briefly in this review (2, 6). These mechanisms provided an initial concept of the early phase of reperfusion injury (2). Mitochondria participate in the activation of programmed cell death (7), either from MPTP or via selective signaling-pathway permeation of the outer mitochondrial membrane [so-called mitochondrial outer membrane permeabilization (MOMP)] (8). Either occurrence can lead to the release of mitochondrial proteins that activate cell death mechanisms, including apoptosis, necrosis, and programmed necrosis (necroptosis) (9). Although most prominent during reperfusion, evidence of activation of programmed cell death is apparent during ischemia (10). This review presents data showing that the ETC is an upstream activator of these processes and examines the sites of damage to cardiac mitochondria during ischemia, with special emphasis on the ETC. The ETC first drives damage to itself, leading to additional damage to other biochemical targets and compartments of mitochondria. In this review, we examine the mechanisms that

translate ischemic mitochondrial injury into cellular damage during ischemia and early reperfusion. Mitochondria are not merely stochastic nodes of oxidative and calcium-mediated injury to the myocyte; dysfunctional mitochondria activate mitochondrial remodeling and cellular reactions that modulate the balance between cell death and recovery. These ideas have therapeutic implications, as discussed below.

NORMAL MITOCHONDRIAL PHYSIOLOGY

Mitochondria are the sites of oxidative metabolism in cardiomyocytes. Cardiac mitochondria exist in two functionally distinct populations: subsarcolemmal mitochondria (SSM) located immediately underneath the plasma membrane and interfibrillar mitochondria (IFM) situated among the myofibrils (11, 12) (**Figure 1a**). In addition to energy production, mitochondria contribute to the regulation of cell function via synthetic pathways and are involved in cell stress pathways. Mitochondrial structure provides compartmentation of these tasks (11) (**Figure 1b**). The outer mitochondrial membrane surrounds the organelle and has among its constituents a voltage-dependent anion channel (VDAC) that provides a route for metabolic substrates (e.g., pyruvate, glutamate, and malate) and nucleotides (e.g., ADP and ATP) to gain entrance to the intermembrane space. The inner membrane is a selectively impermeable barrier with specific transporters and translocases that facilitate the passage of the aforementioned substrates into the matrix, where they are metabolized by enzymes that include those in the tricarboxylic acid (TCA) cycle and fatty acid oxidation, as well as synthetic and antioxidant enzymes. The TCA cycle and fatty acid oxidation generate reduced nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide (FADH₂) for oxidation by the ETC (**Figures 1c** and **2a**).

Cristae, inward folds of the inner membrane, house the ETC and phosphorylation apparatus (**Figure 1b**). The ETC consists of four multiprotein complexes (complexes I–IV) composed of mitochondria- and nuclear-encoded subunits. Each complex contains catalytic and regulatory subunits for a specific reaction sequence for electron transfer (**Figure 1c**). The ETC also contains the mobile electron carriers coenzyme Q and cytochrome *c* (**Figures 1c** and **2**). NADH is oxidized by complex I, leading to the reduction of coenzyme Q, which is then oxidized by complex III, resulting in the reduction of cytochrome *c*. The latter is oxidized by complex IV, transferring the electrons to oxygen to form water. These three complexes actively transport hydrogen ions from the matrix to the cytosolic side of the inner membrane, capturing and storing the energy as a proton gradient. Complex II oxidizes succinate produced in the TCA cycle and reduces coenzyme Q, which enters the ETC at complex III.

The phosphorylation apparatus, located in the inner membrane, consists of complex V (ATPase), a phosphate transporter, and adenine nucleotide translocase (ANT) (11). Complex V, situated in the cristae, harnesses the proton gradient to phosphorylate ADP to ATP. Inorganic phosphate and ADP enter the matrix via inner membrane transporters. These processes couple respiration to phosphorylation, that is, oxidative phosphorylation (OXPHOS). Myocyte energy demand determines the rate of OXPHOS.

DAMAGE TO MITOCHONDRIA DURING CARDIAC ISCHEMIA

Mitochondria sustain progressive injury during cardiac ischemia. Injury progresses both in a regional subpopulation-dependent manner as well as to specific sites in the ETC within both populations of mitochondria. The progression of ischemic injury is more rapid in SSM than in IFM (13). SSM have a decreased capacity for calcium accumulation compared to IFM and are more susceptible to calcium overload-mediated cytochrome *c* release and damage (14). In the canine

SSM: subsarcolemmal mitochondria

IFM: interfibrillar mitochondria

OXPHOS: mitochondrial oxidative phosphorylation

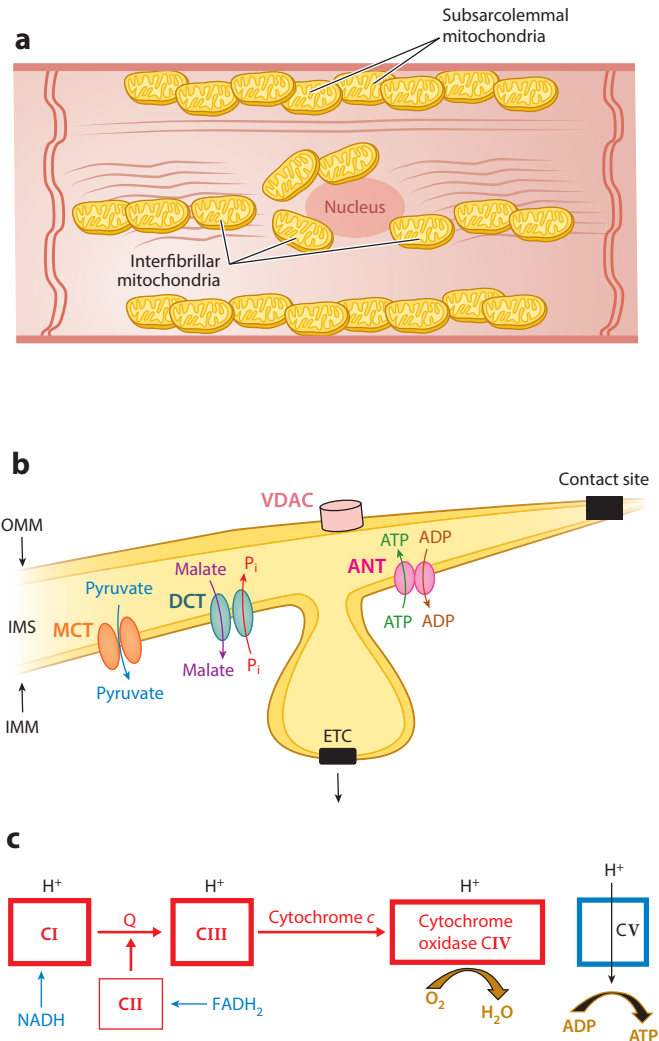


Figure 1

Structure and function of normal mitochondria. (a) The locations of subsarcolemmal and interfibrillar mitochondria in the cardiomyocyte are depicted schematically. The various cellular components are not drawn to scale. (b) Shown are the locations of the voltage-dependent anion channel (VDAC) in the outer mitochondrial membrane (OMM); the electron transport chain (ETC) in the cristae of the inner mitochondrial membrane (IMM) with the adenine nucleotide translocase (ANT), monocarboxylate transporter (MCT), and dicarboxylate translocase (DCT) in the IMM; the intermembrane space (IMS); and contact sites as fusion points of the outer and inner boundary membrane. (c) The ETC. Reduced nicotinamide adenine dinucleotide (NADH) donates an electron to complex I (CI) with flow through to complex IV (CIV) coupled with H⁺ pumping into the IMS at CI, CIII, and CIV. Reduced flavin adenine dinucleotide (FADH₂) feeds electrons into CII with flow to CIV. However, no H⁺ is pumped into the IMS at complex II. H⁺ moves into the matrix coupled with the phosphorylation of ADP to ATP by CV. Other abbreviation: Q, coenzyme Q.

heart subjected to regional ischemia, OXPHOS is decreased to a greater extent in SSM than IFM (15, 16).

Mitochondrial electron transport sustains progressive damage during ischemia (13, 17–19) (**Figure 2a**). OXPHOS is preserved in both SSM and IFM in periods of ischemia of <20 min that do not result in cell death. Oxidation of the complex I substrate glutamate decreases relatively early, by 15 min, in the course of ischemia, whereas the oxidation of the complex II donor succinate decreases as ischemic periods lengthen to 30–60 min (11, 18), suggesting that damage occurs distal to complex I (18). Damage also occurs to the phosphorylation apparatus, including complex V (18) and the adenine nucleotide transporter (20). As ischemia is extended, complex III (19) and cytochrome oxidase (complex IV) are also damaged (11, 13). Following 45 min of ischemia in the rabbit heart, OXPHOS is decreased in SSM, but not in IFM, for all substrates tested, thus localizing the defect to cytochrome *c* and cytochrome oxidase. Mitochondrial respiration remains well coupled with preserved ADP:O ratios and unchanged rates of State 4 respiration. Uncoupled respiration is also decreased, thereby localizing the defect to the ETC (13, 19).

MITOCHONDRIAL MORPHOLOGY WITH ISCHEMIA

Electron microscopy reveals that during ischemia, SSM and IFM remain intact and their appearance is similar to that in nonischemic hearts (**Figure 3a,b**) (13); similar findings occur with isolated SSM and IFM (**Figure 3c,d**). Isolated mitochondria generally appear intact, with slight dilatation of the cristae and swelling of some organelles. Mitochondria isolated following ischemia show only decreased matrix density and minor vacuolization of cristae compared to nonischemic controls. Even after 45 min of ischemia (13, 17), morphological evidence of irreversible injury to myocytes and isolated mitochondria is absent. Dimethyl sulfoxide and acrolein added to fixative speeds its penetration of solid tissues compared to conventional fixatives (19), thus averting prominent morphological changes in ischemic cardiomyocytes (21).

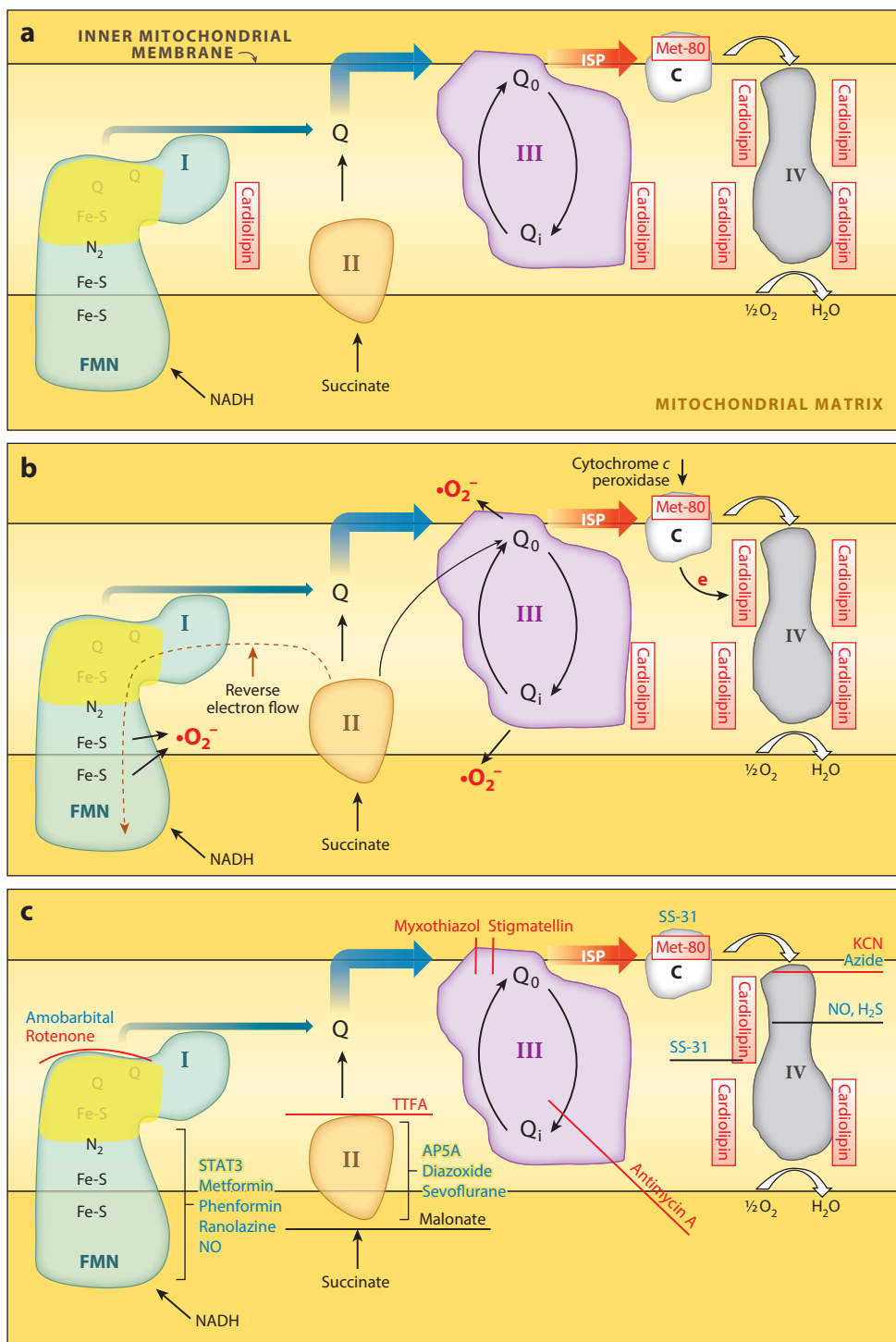
MOLECULAR PATHOLOGY OF ELECTRON TRANSPORT CHAIN DAMAGE

Complex I

Complex I activity decreases during ischemia (18, 19), in both SSM and IFM (22). Complex I activity is also altered by posttranslational modifications, including *S*-nitrosylation (23) and phosphorylation (24). Ischemic damage causes an increase in generation of ROS from complex I (25).

NADH ferricyanide oxidoreductase enzyme activity reflects the activity of NADH dehydrogenase, the initial component of complex I (18, 26). Ischemia does not decrease activity in either SSM or IFM, indicating that damage occurs at more distal sites in the complex (22, 25). In contrast, others have reported a decrease in the NADH dehydrogenase component of the complex (18) due to the loss of the flavin mononucleotide (FMN) coenzyme (**Figure 2a**) (18). An explanation for the different observations is not immediately apparent but may be due in part to differing models of ischemia.

Complex I undergoes conformational change in response to hypoxia and ischemia, shifting from the normal, active A form to the inactive, or dormant, D form when the iron-sulfur clusters and FMN are in the reduced state and the terminal acceptor ubiquinol pool is reduced (27). Conversion to the D form exposes oxidative-sensitive sulfhydryl groups (27), including cysteine 39 of mitochondria-encoded NDUF3 as well as cysteines on NDUF9 and NDUF1 (28). Reversible glutathionylation of these residues occurs as part of an antioxidant defense mechanism that



protects complex I. Reaction with oxidants can trap complex I in the D form, leading to persistent decreased activity. In the absence of oxidation of the sulfhydryl groups, incubation of complex I with substrate leads to the regeneration of the A form and restores activity. Interactions of complex I with mitochondria-localized thioredoxin-2 or glutaredoxin-2 may enhance protection of complex I. The D form is favored, at least during stop-flow ischemia (27, 28). The D form blunts reverse electron transport that generates superoxide (29, 30) and inhibits ROS production via decreased forward flow (27). The role of complex I blockade with rotenone or amobarbital in favoring the A to D transition is the subject of current work. Phenformin, a biguanide, favors the transition of complex I to the D form (31). Metformin in high doses inhibits complex I partially in ischemic hearts and, if given for 5 min at the onset of reperfusion, decreases cardiac damage, as does amobarbital (32–34). Thus, modulation of the A to D transition of complex I may emerge as a key factor in cardioprotection.

ISP: iron-sulfur protein

Complex I produces ROS (26, 35, 36) (**Figure 2b**). Candidate sites for ROS production are FMN in NADH dehydrogenase (37), iron-sulfur cluster N2, and the two tightly bound ubiquinones located distal to the path of electron flow (26). In the baseline state, inhibition of NADH dehydrogenase decreases ROS generation, indicating that the site is distal to the FMN (26), although other investigators previously assigned ROS production to this site (38). Ischemia increases ROS generation from complex I despite preserved NADH dehydrogenase activity (25), localizing this site distal to FMN (26). Rotenone blocks complex I at the ubiquinone acceptor site (39). Inhibition by rotenone increased ROS generation from complex I after ischemia, indicating that the site is proximal to the ubiquinone binding site (26). Based on the use of forward and reverse electron flow (REF) (40, 41), ROS production after ischemia is likely from either the N2 iron-sulfur site or the tightly bound quinones within complex I (26).

Complex III

Complex III activity decreases during ischemia (18, 19). Mammalian complex III contains three catalytic subunits: cytochrome *b*, cytochrome *c*₁, and the iron-sulfur protein (ISP). The ISP contains a 2Fe-2S cluster coordinated by two cysteines and two histidines (42). The contents of cytochromes *b* and *c*₁ were unchanged by ischemia in both SSM and IFM from the rat heart (19). The content of ISP was measured by assessing the electron paramagnetic resonance (EPR) signal generated by the 2Fe-2S cluster in submitochondrial particles from nonischemic controls and from rat hearts following ischemia, which decreased the *g* 1.79 and *g* 1.89 EPR signals of the ISP dramatically in

Figure 2

Sites of ischemia-mediated damage to electron transport. (a) Sites of ischemia damage to the electron transport chain (ETC) complexes. (b) Sites within these complexes where electrons leak to form superoxides and subsequently other reactive oxygen species, including hydrogen peroxide (H₂O₂). (c) Sites of action of classical chemical inhibitors (*red*) and potential therapeutic agents that modulate the ETC (*blue*). Potential therapeutic modulators of complex I include amobarbital acting at the quinol binding site (39), SNO donors (99), and nitric oxide (NO) (100) which likely interact with iron-sulfur (Fe-S) centers, and STAT3 (121). Other compounds that inhibit complex I partially and that have been reported to result in cardioprotection include metformin (31), phenformin (31), and ranolazine (97) (specific sites not depicted). Malonate competes for oxidation of succinate by complex II (29), whereas AP5A (60), diazoxide (200), and sevoflurane (201) alter other mechanisms. The peptide SS-31 binds to cardiolipin and also inhibits cytochrome *c* peroxidase (83, 86, 202–204). Other abbreviations: AP5A, diadenosine pentaphosphate; C, cytochrome *c*; FMN, flavin mononucleotide; I, II, III, and IV, complexes I, II, III, and IV; ISP, iron-sulfur protein; NADH, nicotinamide adenine dinucleotide; Q, coenzyme Q; STAT3, signal transducer and activator of transcription 3; TTFA, thenoyltrifluoroacetone.

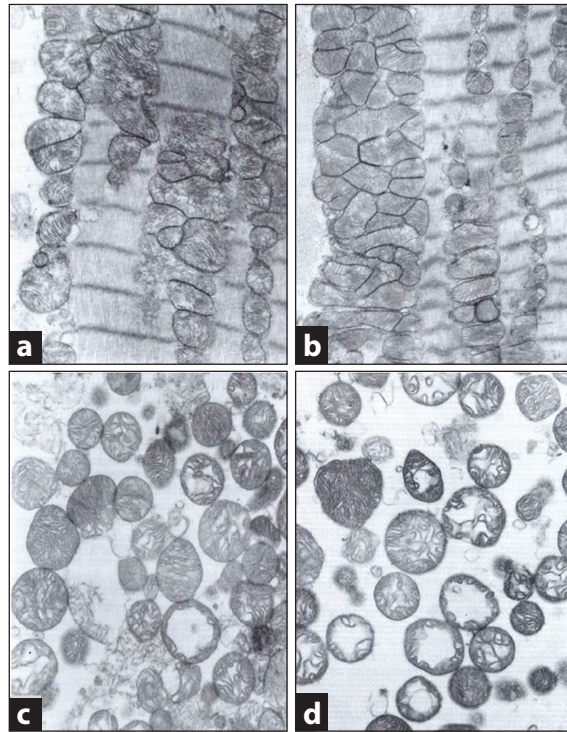


Figure 3

Mitochondrial morphology is preserved following ischemia alone. Electron micrographs of in situ subsarcolemmal mitochondria (SSM) and interfibrillar mitochondria (IFM) in nonischemic controls (*a*) and myocytes following 45 min of stop-flow ischemia (*b*) in the rabbit heart. Mitochondria are intact and, following ischemia, exhibit minimal change, confirming their morphological integrity at the end of the ischemic period. Micrographs of isolated SSM from nonischemic control (*c*) and ischemic hearts (*d*) confirm purity of the isolated mitochondrial population and reinforce the paucity of ischemia-induced damage to these organelles. Figure courtesy of American Physiological Society, copyright 1997 (13).

both SSM and IFM (19). Ischemia did not decrease the content of ISP peptide despite a marked decrease of the EPR signal of the ISP; the other 11 subunits of the complex, including the redox centers cytochrome *b* and ϵ_1 , were unaltered (19) (**Figure 2a**). Complex III remains a key site for oxidant generation during and following ischemia. Most of the ROS generated at complex III are from the Q_o (outer quinol binding site) oxidation center, although the Q_i (quinol site internal) oxidation center also contributes (43, 44). Reduced coenzyme Q binds to the Q_o center on cytochrome *b* and undergoes oxidation, providing the site of entry for electrons into complex III. This oxidation generates two electrons with the transfer of one electron to ISP and the other electron to the Q_i center on the matrix side of cytochrome *b* (**Figure 2a**). Ischemic damage to complex III allows adequate residual electron flow into the complex for ROS production, probably because complex III exists as a dimer. Inactivation of one of the Q_o sites in a dimer facilitates ROS generation from complex III Q_i centers (45) (**Figure 2b**).

Transient, reversible blockade at the Q_o site of complex III is an appealing therapeutic intervention. Myxothiazol and stigmatellin, irreversible inhibitors and mitochondrial poisons, attenuate ROS production from complex III (46). In contrast, a reversible inhibitor of the Q_o site has the potential to inhibit ROS production and blunt reperfusion injury.

Complex IV–Cardiolipin

Activity of Complex IV, composed of 13 peptide subunits, requires the integrity of catalytic subunits (47) and regulatory and structural subunits (48). Complex IV is located in respirasomes with complexes I and III that optimize channeling of respiratory substrates (49). Ischemia does not deplete or inactivate subunit peptides (13). Cardiolipin is the only phospholipid that is decreased in content; this loss in SSM occurs over an interval that parallels the decrease in OXPHOS through cytochrome oxidase (17), providing a mechanism for the complex IV defect (**Figure 2a**). Mitochondrial cardiolipin content also decreases during ischemia in isolated rat heart (50, 51) and in vivo canine heart (52, 53). Fusion of isolated rat heart mitochondria with cardiolipin-containing liposomes restores cytochrome oxidase activity (51).

Loss of cardiolipin from SSM during ischemia plays an important role in mitochondrial damage. The high content of oxidatively sensitive linoleic acid (C18:2) acyl groups (54) renders cardiolipin susceptible to oxidative damage, but the unaltered composition of cardiolipin following ischemia excludes the presence of oxidatively altered acyl groups (17). However, oxidatively generated peroxy groups in cardiolipin are unstable, may decompose rapidly, and lead to cardiolipin destruction (55). Alternatively, oxidized acyl residues may react directly with proteins, leading to covalent phospholipid-protein complexes that render cardiolipin no longer extractable by organic solvents (56). Thus, oxidative processes cannot be excluded merely by the failure to detect oxidized cardiolipin.

Complex II

Complex II is relatively resistant to ischemic damage (13). Its activity decreases in rat heart mitochondria during prolonged global ischemia (**Figure 2a**) and decreases further after reperfusion (57). This decreased activity is related to a reduction of *S*-glutathionylation in the 70-kDa flavin protein (58) and increased oxidation and nitration of complex II, impairing its interaction with complex III (59). Modulation of complex II activity decreases cardiac injury during I/R and contributes to opening of the mitochondrial K_{ATP} channel (60) and inhibition of ROS generation (61). Defects in mammalian complex II increase ROS production, likely from auto-oxidation of $FADH_2$ or $FADH^{\bullet-}$ semiquinone (62).

In intact heart mitochondria, ROS can be generated through REF from complex II to complex I using succinate as substrate (41, 61) (**Figure 2b**). This generation is affected by the inner membrane potential ($m\Delta\Psi$) (63) and by inhibitors of complex I (41) and complex II (61). With succinate as a substrate, inhibition by oligomycin increases REF-mediated ROS generation (61) by increasing $m\Delta\Psi$. In contrast, depolarization of the inner membrane inhibits REF-mediated ROS generation completely (41, 63). Following prolonged ischemia, $m\Delta\Psi$ is depolarized in heart mitochondria (25, 41, 64). REF-mediated ROS generation can still occur in the heart following mild ischemia (29) that may not have led to depolarization of the inner membrane. Preventing REF-mediated ROS production decreases cardiac injury during I/R (29, 30). The role of REF-mediated ROS generation in cardiac injury might depend on the degree of ischemic damage sustained by the mitochondria.

Therapies to decrease REF-mediated ROS can be approached in several ways. Blockade of complex II with malonate or diadenosine pentaphosphate (60) has clinical potential, but thenoyltrifluoroacetone, an irreversible inhibitor, has little utility. 3-Nitropropionic acid, an inhibitor of complex II, produces ROS and can result in cytoprotection (65). As noted above, relative uncoupling of $m\Delta\Psi$ will also attenuate REF. Safe approaches to uncouple respiration have been described (66, 67).

Cytochrome *c*

Ischemia results in the loss of cytochrome *c* from mitochondria (7, 10, 13) into the cytosol (7). Concomitant with cardiolipin loss, cytochrome *c* decreases in SSM at 30 min of ischemia (17). Unlike IFM, SSM *in vitro* exhibit a greater tendency to release cytochrome *c* (12) and to sustain rapid progression of ischemic damage (13), including the loss of cytochrome *c* (17). SSM are more sensitive to cytochrome *c* release by elevated external calcium (14) and thus more likely to activate cardiomyocyte death programs.

Cardiolipin provides binding sites on the inner membrane for cytochrome *c* (68), so a decrease in its content favors the loss of cytochrome *c*, a process that occurs during I/R (68). Thus, the decrease in mitochondrial content of cytochrome *c* is linked to the depletion of cardiolipin, followed by permeabilization of the outer membrane, either via proapoptotic peptides (69) or by MPT (10).

MITOCHONDRIAL INJURY DURING ISCHEMIA-ELECTRON TRANSPORT CHAIN-DRIVEN INJURY

Ischemic damage to mitochondria is mediated at least in part by mitochondria themselves (70). Blockade of the ETC with rotenone or amobarbital protects them against ischemic damage. When the ETC in the intact heart is inhibited with rotenone immediately before ischemia, the content of cardiolipin, cytochrome *c*, and OXPHOS through cytochrome oxidase is preserved (70) and ischemic damage is markedly attenuated (**Figure 2c**). It is not surprising that preservation by rotenone of cardiolipin during ischemia preserves cytochrome *c* content.

Amobarbital (**Figure 2c**), a short-acting barbiturate, inhibits complex I reversibly at the rotenone site (39). Amobarbital treatment of the isolated rat heart just before ischemia protected OXPHOS, including cytochrome oxidase; preserved cytochrome *c* content during subsequent ischemia; and protected respiration with glutamate, a complex I substrate (71). Amobarbital washes out of mitochondria rapidly during isolation; its reversible blockade of respiration at complex I protects complex I from ischemic damage (71). Preservation of OXPHOS with substrates for complex III and IV shows that the distal ETC is also protected. Thus, blockade of electron transport at complex I using two chemically dissimilar compounds immediately before ischemia protects against ischemic damage to the ETC.

SITES IN THE ELECTRON TRANSPORT CHAIN THAT GENERATE ISCHEMIC DAMAGE

Historically, the study of oxidative damage to the myocardium focused on the reperfusion phase. Oxidative damage also can occur during ischemia, as oxygen remains available early in the process (72) because cytochrome oxidase does not consume all of the residual oxygen rapidly. In line with this observation, ROS production increases during global ischemia (73). Reduced electron flow occurs owing to the decreasing concentration of oxygen (74) as well as the ETC damage (13, 18, 19) (**Figure 2b**). With complete anoxia, ROS production ceases, paradoxically attenuating injury in isolated cardiac myocytes (75).

ROS production and Ca^{2+} loading were lessened during ischemia in guinea pig hearts treated with amobarbital (73). Blocking electron transport during ischemia preserves respiratory function in isolated mitochondria (71), decreases ROS production, and reduces Ca^{2+} accumulation in intact hearts (73). The importance of ROS production by mitochondria is supported by cardiac protection resulting from treatment with mitochondria-targeted antioxidants (76, 77).

Mitochondria are the primary source of ROS production during ischemia (75, 78) (**Figure 2b**). Superoxide production during simulated ischemia in cardiac myocytes is decreased by blockade of electron transfer into complex III (75, 78), thereby lessening the amount of molecular oxygen that can form superoxide (46). Antimycin A, an inhibitor of complex III, blocks distal to the Q_o site of the complex and augments the net production of ROS from complex III (46) (**Figure 2c**). Inhibition of cytochrome oxidase increases ROS generation from upstream redox sites (78).

We investigated the role of electron transport distal to the Q_o site of complex III in the damage to cardiolipin during ischemia. Treatment with antimycin A before ischemia preserved the content of cardiolipin in SSM and decreased the loss of cytochrome *c* in a fashion similar to the protection with rotenone (70), suggesting that ischemic damage occurs distal to the Q_o site of complex III.

Cytochrome oxidase might produce ROS (79) and toxic NO from nitrates during hypoxia (80). In isolated rabbit hearts that had undergone 30 min of global stop-flow ischemia, we blocked the ETC either at complex I using amobarbital or at cytochrome oxidase using azide before ischemia. Azide treatment (**Figure 2c**) did not protect against ischemia-induced decreases in respiration (81) or in cytochrome *c* in SSM. In contrast, blockade of complex III at the Q_i site attenuated the depletion of cardiolipin and loss of cytochrome *c* during ischemia (70). These observations localize the site that depletes cardiolipin and cytochrome *c* to the ETC segment encompassing the complex III Q_i site and cytochrome *c*.

Cardiolipin interacts with cytochrome *c* via nonionic (82) and electrostatic mechanisms to bind it to the inner mitochondrial membrane (68). In pathological settings (e.g., oxidative stress), cytochrome *c* undergoes a change in tertiary structure (82). Oxidation of a key methionine residue (Met-80), a ligand of the heme group of cytochrome *c*, occurs in concert with the change in its tertiary structure (83). The iron coordination in the heme of cytochrome *c* is disrupted (83, 84), allowing H_2O_2 to interact with the heme iron, in turn facilitating electron transfer to and peroxidation of acyl groups of cardiolipin (84) (**Figure 2b**).

Formation of the cytochrome *c* cardiolipin peroxidase results in a marked shift in redox potential, with cytochrome *c* being unable to be reduced by complex III (84) and with blockade of electron flow into complex III. Once ischemic damage to the ETC forms the peroxidase, the sites of the ETC for ROS production likely shift from complex III to complex I. An increase in intramitochondrial ROS generation, coupled with the electron flow into the cytochrome *c* segment, is required to oxidize Met-80 (85). Following I/R, methionine oxidation in cytochrome *c* is increased. Thus, oxidized cytochrome *c* is an intriguing therapeutic target.

Short peptides consisting of alternating aromatic and charged amino acids cross cell membranes and are incorporated into mitochondria. Such peptides, including SS-31, are inserted into mitochondrial membranes and protect cardiolipin from oxidation by inhibiting cytochrome *c* cardiolipin peroxidase (86). SS-31 reduces infarct size in I/R rodent models, supporting a role for cytochrome *c* cardiolipin peroxidase in injury during I/R (87). Clinical trials are in progress to test whether SS-31 can ameliorate heart failure and genetic mitochondrial disease and reduce infarct size (88).

The p66^{shc} protein, located in the mitochondrial intermembrane space, generates H_2O_2 using electrons transferred from reduced cytochrome *c* (89) and activates apoptosis (90). Durations of ischemia that lead to cell death are associated with phosphorylation and migration of p66^{shc} from the cytosol to mitochondria (91). p66^{shc} is an alternative to complex III for ROS production during ischemia (85). Complex III or p66^{shc} can potentially generate ROS concomitant with electron flow through cytochrome *c* to form the peroxidase to oxidize cardiolipin, leading to decreased content of the latter (17).

Table 1 Cardiac ischemia-mediated mitochondrial injury in different species

Species	Time course of ischemia	Mitochondrial damage	Advantages	Disadvantage
Mouse (34, 116, 125)	Mixed population of mitochondria at 25–30 min ischemia	OXPHOS ↓, cytochrome <i>c</i> ↓, AIF ↓, MPTP ↑	Genetic models; mechanism testing	Small heart size
Rat (19, 22)	SSM ≈ IFM at 30 min ischemia	OXPHOS ↓, cytochrome <i>c</i> ↓, AIF ↓, cardiolipin ↓, MPTP ↑	Commonly used in vivo and in vitro	Fast heart rate
Guinea pig (91)	SSM injury at 30 min ischemia; IFM spared	p66 ^{shc} phosphorylation ↑	Larger heart than rat	Fast heart rate
Rabbit (17)	SSM injury at 30 min ischemia; IFM spared	OXPHOS ↓, cytochrome <i>c</i> ↓, cardiolipin ↓, MPTP ↑	Slow heart rate; more similar to human	Slow heart rate
Canine (53)	SSM injury at 60 min ischemia; IFM spared	OXPHOS ↓, CPT-1 ↓	First large animal model	Expensive, highly sentient animal model
Pig (124)	SSM injury at 90 min ischemia; IFM spared	OXPHOS ↓, MPTP ↑	Mimic of human model	Expensive

Abbreviations: AIF, apoptosis-inducing factor; CPT-1, carnitine palmitoyl transferase-1; IFM, interfibrillar mitochondria; MPTP, mitochondrial permeability transition pore; OXPHOS, oxidative phosphorylation; SSM, subsarcolemmal mitochondria.

MITOCHONDRIAL DAMAGE OCCURS PREDOMINANTLY DURING ISCHEMIA

During ischemia of the isolated perfused rabbit heart (**Table 1**), SSM lose cardiolipin and cytochrome *c*, with a decrease in cytochrome oxidase OXPHOS. Reperfusion does not lead to additional damage in the distal ETC (92). OXPHOS through cytochrome oxidase and the content of cytochrome *c* does not decrease further during reperfusion. Thus, impairment of cardiolipin, cytochrome *c*, and cytochrome oxidase occurs during ischemia rather than during reperfusion. Decreased cardiolipin content leads to mitochondrial release of cytochrome *c* (68), decreasing respiration and activating programmed cell death (7). The decrease in electron flux through cytochrome oxidase enhances ROS production from proximal redox centers in the ETC during reperfusion (93). Complexes I and III are key sites that generate ROS that induce mitochondrial damage and myocyte injury during I/R (36, 93, 94).

In sum, ischemic damage to mitochondria leads to ROS production (78), favors onset of MPTP (7), and results in the release of cytochrome *c* (7), all of which are mechanisms of mitochondria-driven cell death during reperfusion (1, 93) (**Figure 4**). Mitochondrial self-injury during ischemia is thus a major mechanism in cardiomyocyte damage during I/R.

CARDIAC PROTECTION FROM ATTENUATION OF MITOCHONDRIAL-DRIVEN MITOCHONDRIAL INJURY

If the ETC remains intact and essentially undamaged at the onset of reperfusion, will myocyte injury occur? Blockade using amobarbital protects electron transport during ischemia (22, 70), supporting the idea that disruption of the ETC is the major source of mitochondrial damage (93). Other studies have documented that mitochondria, when free of ischemic damage at the onset of reperfusion (71), largely avoid cardiac injury (22). Amobarbital treatment (**Figure 2c**) preserves the rate and coupling of OXPHOS in both SSM and IFM following reperfusion (22). Thus, protection of the ETC by reversible blockade during ischemia is carried forward into reperfusion. Reversible

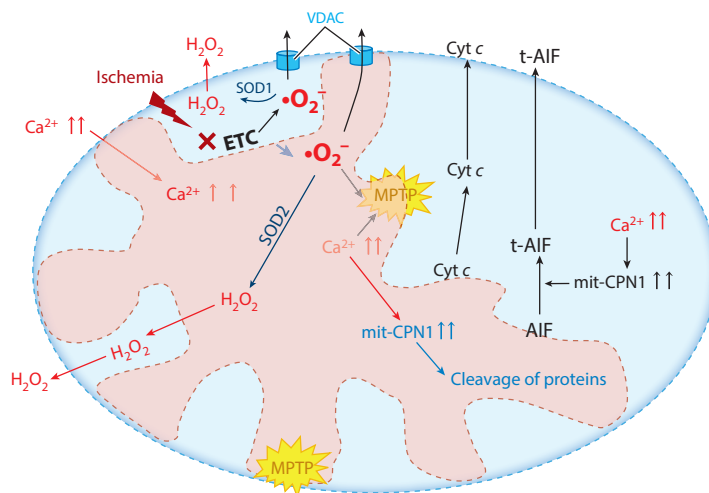


Figure 4

Mechanisms of mitochondrial-driven injury that result from ischemic damage to mitochondria, especially the mitochondrial electron transport chain (ETC). These include activation of the mitochondrial permeability transition pore (MPTP) and permeabilization of the outer mitochondrial membrane, leading to release of cytochrome *c* (Cyt *c*) and activation of mitochondrial calpains (mit-CPN1), which leads to the cleavage and release of truncated apoptosis-inducing factor (t-AIF) and the export of reactive oxygen species generated by the ETC by the voltage-dependent anion channel (VDAC), including mitochondrial contact sites. Antioxidant systems include superoxide dismutase 2 (SOD2) in the mitochondrial matrix and superoxide dismutase 1 (SOD1) in the intermembrane space. Superoxide ($\bullet\text{O}_2^-$) in the mitochondrial matrix is converted to hydrogen peroxide (H_2O_2) by SOD2, whereas superoxide in the intermembrane space is converted by SOD1 to H_2O_2 .

blockade of electron transport during ischemia protected complex I (22) as well as the distal ETC, with preserved respiration through complex III and cytochrome oxidase and improved retention of cytochrome *c* (22).

Several mechanisms may explain why reperfusion of cardiomyocytes that contain mitochondria with essentially normal electron transport decreases cardiac injury (**Figure 4**). First, ROS production from both complex I and complex III is decreased (22). Preservation of respiratory function thus decreases mitochondrial production and release of ROS during reperfusion. Second, blockade of electron transport during ischemia enhances retention of cytochrome *c* during reperfusion (22), probably owing to maintenance of inner mitochondrial membrane integrity (17, 22, 68). Cytochrome *c* delocalized from a damaged inner membrane is released from mitochondria when outer membrane permeability increases (68). Electron transport blockade during ischemia preserves outer membrane integrity after reperfusion (22).

Early reperfusion after amobarbital treatment that led to retained mitochondrial function is associated with less mitochondrial ROS production and Ca^{2+} loading (73). A decrease in ischemic damage to the ETC preserves the antiapoptotic signaling from mitochondria (95) and decreases susceptibility to the onset of MPTP (64), retaining inner and outer membrane integrity. Blockade of electron transport before ischemia markedly reduces infarct size and improves contractile recovery during reperfusion (22, 73, 96). Protection of cardiac mitochondria during ischemia is carried forward into the early reperfusion period and supports the hypothesis that mitochondrial damage occurs predominantly during ischemia and mitochondria-mediated cardiomyocyte injury occurs largely during reperfusion.

Reversible inhibition of complex I at the rotenone site has potential therapeutic utility. Blockade at this site attenuates REF (40, 41) and decreases ROS production from complex III (36). Amobarbital has limited utility. Rotenone, an irreversible inhibitor, is toxic during the reoxygenation of reperfusion. Other approaches to attenuate complex I activity reversibly include intracellular acidification at the onset of reperfusion (34), high-dose metformin (32), ranolazine (97), Mito-SNO (98), 3-mercaptopropionylglycine (99), and nitrite (100). Metformin and ranolazine are clinically used drugs.

MITOCHONDRIAL PERMEABILITY TRANSITION DURING REPERFUSION

MPTP are nonselective pores located in the inner mitochondrial membrane (2). Opening of MPTP depolarizes $m\Delta\Psi$, impairing OXPHOS and ATP production. MPTP opening, a pathological event, results in an influx of water and solutes into the mitochondrial matrix, causing mitochondrial swelling and eventual rupture of the outer membrane, which in turn leads to the loss of intermembrane space proteins, including cytochrome *c* and apoptosis-inducing factor (AIF). The release of cytochrome *c* and AIF activates caspase-dependent and caspase-independent cell death programs, leading to apoptosis (101). The precise structure of MPTP is unclear. Regulation of MPTP opening is related to cyclophilin D within the matrix (102). Proteins of the inner and outer mitochondrial membrane include VDAC and ANT, which were considered to be MPTP components, but genetic studies do not support this idea (103). Complex V is reportedly a component of the MPTP (104), and I/R alters complex V subunits (104, 105).

The role of MPTP opening in I/R injury has been reviewed extensively (106). The opening of MPTP can occur during ischemia (7) and is prominent during reperfusion (107). Increased calcium availability during reperfusion enhances mitochondrial calcium loading (73) and, in concert with mitochondrial oxidative damage, leads to MPTP opening (107). As discussed above, ROS production and calcium loading during reperfusion are lessened when mitochondrial function remains normal during reperfusion (73); MPTP opening during early reperfusion is decreased when mitochondria are protected by blockade of electron transport during ischemia (64). Based on their greater sensitivity to calcium-mediated damage (14), MPTP during reperfusion may largely involve SSM. MPTP is not necessarily irreversible during reperfusion, as closure of the MPTP is enhanced by pyruvate oxidation (6). Inhibition of MPTP opening decreases cell death (6), supporting the idea that MPTP contributes to cardiac injury.

The importance of mitochondrial calcium loading during early reperfusion is supported by the finding that interventions to attenuate myocardial calcium loading result in cardiac protection. These pharmacological treatments include blockade of the mitochondrial calcium uptake uniporter using ruthenium red (108, 109), blockade of sodium-hydrogen exchange (110), and blockade of sodium-calcium exchange (111).

Cyclosporin A binds to cyclophilin D and decreases markedly the susceptibility of mitochondria to MPTP, thus leading to a decrease in cell death. Cyclosporin A protects myocardium when given either before ischemia or at the onset of reperfusion (112). Initial human trials showed a benefit of cyclosporin A given at the onset of reperfusion (113), supporting the clinical importance of mitochondria as generators of cardiac reperfusion injury. As measured in the intact heart (73), protection of mitochondrial function during ischemia by reversible inhibition of electron transport (71) reduces mitochondrial calcium loading during reperfusion, decreases oxidant production (22), and prevents MPTP opening (64). In sum, ischemia-damaged ETC is a key factor in inducing MPTP opening during reperfusion.

MODULATION OF ISCHEMICALLY DAMAGED MITOCHONDRIA DURING REPERFUSION PROTECTS AGAINST CONSEQUENCES OF ISCHEMIA-INDUCED MITOCHONDRIAL DAMAGE

At the onset of reperfusion, transient and reversible blockade of the proximal ETC decreases myocardial injury (33, 93, 114), although the extent of protection is less than if intervention occurs before ischemia (114). Thus, early reperfusion is a critical window of opportunity for interventions to attenuate electron transport–dependent cardiac injury even though ischemia-mediated mitochondrial damage has already occurred (64, 115). Ischemic damage to the distal ETC emerges as a link between ischemia and the mitochondrial-driven myocyte injury that occurs during reperfusion (**Figure 4**).

In addition to decreased OXPHOS, reperfusion leads to the loss of key signaling proteins located within mitochondria (7, 116) (**Figure 4**). Cytochrome *c* and AIF, located within the intermembrane space (117), contribute to the function of the ETC (101). The release of these proteins from mitochondria in response to MPTP or MOMP triggers programmed cell death networks. Release of cytochrome *c* promotes caspase-mediated cell death programs (7), whereas release of AIF enhances caspase-independent cell death programs (117). Interventions applied at the onset of reperfusion thus might be able to block the loss of these proteins from mitochondria and, consequently, programmed cell death (101).

STAT3: signal transducer and activator of transcription 3

RECRUITMENT OF TRANSCRIPTION FACTORS TO MODULATE ELECTRON TRANSPORT

Mitochondria-localized transcription factors can modulate ETC function and alter mitochondrial function by interacting with the apoptotic machinery or changing mitochondrial RNA expression (118). Signal transducer and activator of transcription 3 (STAT3), the best-studied transcription factor during ischemia, regulates the expression of genes that encode proteins involved in cellular responses to stress (119) and protection from injury (120). Mitochondrial STAT3 regulates electron transport by nontranscriptional mechanisms (121) and interacts with matrix-localized cyclophilin D, an MPTP component (122). Genetic deletion of STAT3 impairs complexes I and II (123) and increases sensitivity to calcium overload that leads to MPTP opening (122).

Cardiac overexpression of a mitochondrial-targeted, transcriptionally inactive form of STAT3 (MLS-STAT3E) leads to persistent, partial blockade of complex I, which protects it from ischemic damage (**Figure 2c**), decreases ROS production following ischemia, and enhances mitochondrial retention of cytochrome *c* (121). MLS-STAT3E response during ischemia is reminiscent of approaches using pharmacological blockade of complex I (1), which protects mitochondria and the myocardium (124). Overexpression of MLS-STAT3E can reduce cardiac injury after I/R (125). In line with these observations, cardioprotection in an in vivo pig model of regional myocardial I/R (considered similar to I/R in humans) leads to phosphorylation of mitochondrial STAT3, protection of complex I, and increased calcium tolerance (124). Enhancing mitochondrial STAT3 activation and content during ischemia or early reperfusion may be a way to harness intrinsic protective mechanisms. Activation of STAT3 to alter mitochondrial metabolism during ischemia or at the time of reperfusion using leptin (126), oncostatin M (127), or even tumor necrosis factor- α (128) is a future possibility.

PRECONDITIONING AND MITOCHONDRIAL FUNCTION

Ischemic preconditioning (IPC), wherein brief periods of ischemia followed by reperfusion are applied immediately before sustained ischemia, reduces infarct size by decreasing apoptosis and

necrosis (129). The early phase of IPC protection results from activation of G protein-coupled receptors (129) that trigger cardioprotective signaling pathways (129, 130) and ultimately inhibit MPTP (131, 132). In addition, signaling ROS generated in mitochondria during IPC activate mitochondrial ATP-dependent potassium (m-K_{ATP}) channels, which prevent mitochondrial Ca⁺² overloading (133). The early phase of preconditioning involves signaling that impinges on mitochondria to modulate function and cardioprotection, including preservation of OXPHOS during reperfusion. IPC and blockade of electron transport thus achieve cardioprotection by different mechanisms that modulate mitochondrial metabolism during ischemia. Therapeutic approaches based on IPC mechanisms are limited by the inability to predict the onset of coronary thrombosis. Anesthetic preconditioning, especially with sevoflurane, activates these mechanisms, exhibits efficacy, and is likely applicable to surgical settings when the onset of myocardial ischemia is known (111, 134, 135). The repurposing of agents that elicit IPC responses as chronic, preemptive therapeutics in high-risk patients merits consideration. Diazoxide (136) and nicorandil (137) are two clinically used agents that elicit IPC responses.

REMOTE CONDITIONING

Protection against I/R injury to the heart might be provided by applying cycles of I/R to seemingly unrelated vascular beds. Such remote conditioning produces changes in blood and neurons that are transmitted to the heart. The precise mechanisms by which this protection is conferred have not been identified in their entirety, although some are known (see 138–140). This protection appears to be independent of the time when cardiac ischemia will occur or has occurred (141–143). Proposed triggers include small-molecule receptor agonists (144–146). Researchers have proposed humeral, neural, exosomal, and cellular mechanisms to explain the communication between distant organs and the myocardium (141, 147, 148). Neural pathways are increasingly supported as an obligate mediator in the protective signaling (149, 150). Remote conditioning maintains the integrity of mitochondrial structure, attenuates loss of cytochrome *c*, and inhibits MOMP (151).

In the setting of coronary artery bypass grafting, the benefits of remote conditioning have been disappointing (152–158). However, there is still substantial enthusiasm for remote conditioning in myocardial protection in the treatment of coronary occlusion because of its ease of application, minimal adverse effects, and low cost. Notably, remote conditioning in ST segment myocardial infarction has had some encouraging results (157, 158). Because numerous factors that are released as the result of conditioning have been identified, the possibility exists that pharmacological mimetics could be developed; such agents would enhance the practicality and effectiveness of conditioning as therapy.

MITOCHONDRIA-LOCALIZED CALPAINS AND APOPTOSIS-INDUCING FACTOR: SENSING MITOCHONDRIAL CALCIUM OVERLOAD INJURY

Calpains are a family of Ca²⁺-dependent cysteine proteases generally considered to be localized in the cytoplasm; this includes calpain I (μ-calpain), which is activated in the presence of micromolar (μM) calcium (159, 160) and is regulated by the endogenous inhibitor calpastatin (160, 161). Calpain 10 and μ-calpain localize to mitochondria (162, 163), the former in the matrix (164). AIF must be detached from the inner mitochondrial membrane via cleavage to truncated AIF (t-AIF) by mitochondrial-localized μ-calpain (116, 162) with subsequent release when the outer membrane is breached by MPTP or MOMP (**Figure 4**). Mitochondrial calpains constitute a calcium-responsive signaling system that activates caspase-independent cell death programs.

Activation of mitochondrial calpains appears to increase the susceptibility to MPTP. Mitochondrial calpain activity is inhibited by intracellular acidic pH, the endogenous inhibitor calpastatin (162), and pharmacological inhibitors (165), all potential means of reducing caspase-independent cell death (118). Cell-permeable calpain inhibitors (e.g., MDL-28170) have a favorable short-term toxicity profile, do not impair cardiac contractile function, and are chemical templates for the development of clinical agents (166).

Drp1: dynamin-related protein 1

MITOCHONDRIAL DYNAMICS

Although mitochondria can be modulated with ischemia-damaged ETC to reduce cardiomyocyte injury during early reperfusion (33, 93, 114), longer-term cardiomyocyte survival and recovery from ischemia require that the damaged mitochondria be repaired, removed, or replaced (**Figure 5**). Mitochondrial repair or removal involves fusion, fission, and autophagy. Mitochondrial fusion proteins include mitofusin 1 (Mfn1), Mfn2, and optic atrophy 1 (OPA1). Mitochondrial fission proteins include dynamin-related protein 1 (Drp1), mitochondrial fission protein 1 (Fis1), mitochondrial fission factor (MFF), and mitochondrial dynamics proteins of 49 and 51 kDa (MiD49/51) (167–169).

Mfn1 and Mfn2 are on the outer mitochondrial membrane and play a key role in regulating membrane fusion. Mfn2 is found on the endoplasmic reticulum (ER) and mitochondria-associated membranes (170). In addition to mitochondrial fusion, Mfn2 is involved in calcium signaling between the ER and mitochondria (171). Fusion of the inner mitochondrial membranes is regulated by OPA1, a GTPase present in these membranes. Mfn1 is required for OPA1 to induce mitochondrial fusion (172).

Drp1, a cytosolic protein, is essential for mitochondrial fission. Translocation of Drp1 from the cytosol to mitochondria is the initial step in fission. Drp1 is activated by phosphorylation (173). Activated Drp1 undergoes oligomerization and translocation to mitochondria (173). Fis1, MFF, MiD49, and MiD51 also participate in facilitating the translocation of Drp1 to mitochondria (167).

MITOCHONDRIAL FISSION AND FUSION DURING ISCHEMIA-REPERFUSION

The impairment of mitochondrial dynamic change contributes to cardiac injury during I/R (8, 167). Pharmacological inhibition of Drp1 using mdivi-1 increases the number of elongated IFM with decreased myocardial infarct size in the murine heart following I/R (174). Mfn2 content decreases in the rat heart following I/R, but the translocation of Drp1 to mitochondria increases, accompanied by decreased Mfn1 and Mfn2 and increased Fis1 and Drp1 (173). Thus, I/R favors fission. Drp1 inhibition, using adenoviral overexpression of a dominant-negative Drp1K38A, decreases cardiac injury (175). Nitrite treatment modulates mitochondrial morphology via protein kinase A-dependent phosphorylation of Drp1 that inhibits fission (175). In cardiomyocytes, H₂O₂ treatment increases fission and apoptosis and enhances MFF expression. Downregulation of MFF with siRNA inhibits mitochondrial fission and cell death. Increased MFF expression is also found in heart following I/R (176). These results support the idea that inhibition of mitochondrial fission protects myocytes during I/R. The role of fission and fusion in ischemia has not been studied, nor has protective intervention during early reperfusion with blockade of electron transport to enhance these processes.

REMOVAL BY MITOPHAGY

Mitophagy (mitochondrial autophagy) sequesters and eliminates damaged mitochondria (8, 177, 178) (**Figure 5**). Inhibition of mitophagy during I/R increases cardiac injury, implicating

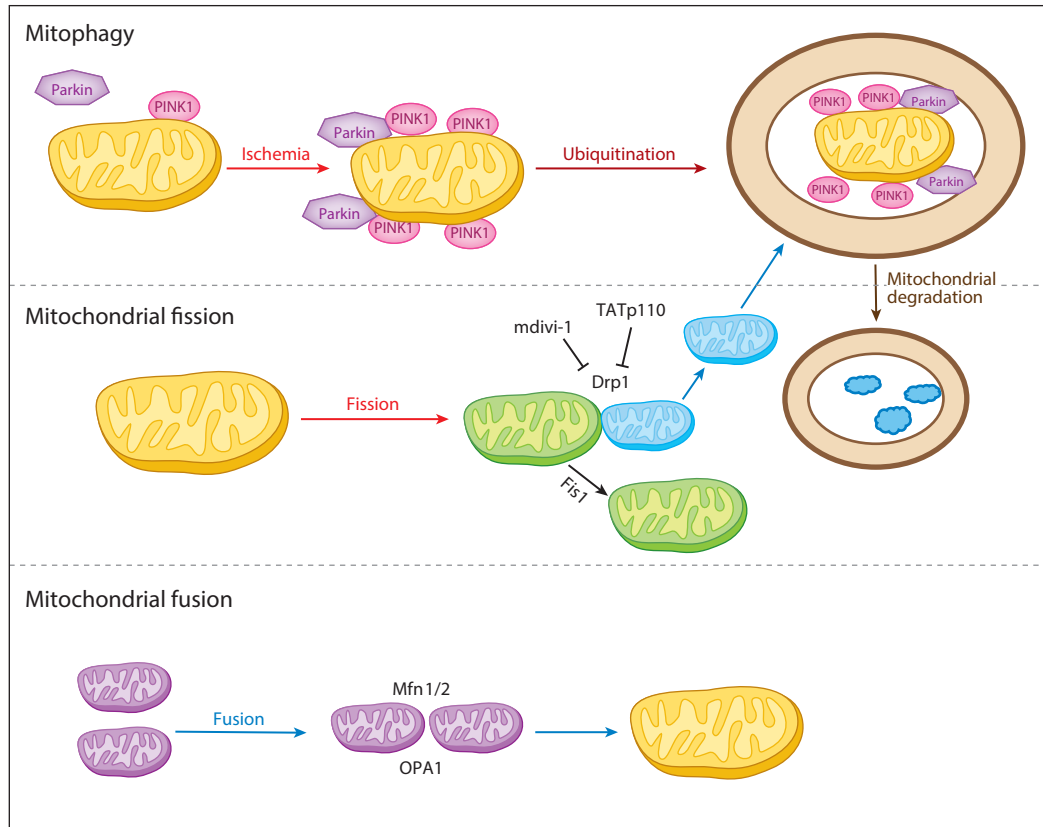


Figure 5

Mitochondrial dynamics. Mitophagy (mitochondrial autophagy) is mediated by Parkin and PINK1. Mitochondrial fission with Drp1 and Fis1 is a key component of the division of mitochondria. Drp1 can be inhibited by mdivi-1 and TATp110 peptides. Mitochondrial fusion utilizes the outer mitochondrial membrane Mfn1/2 and the inner membrane OPA1. Fission of damaged mitochondria can lead to destruction of the daughter organelles by mitophagy. Abbreviations: Drp1, dynamin-related protein 1; Fis1, mitochondrial fission protein 1; Mfn1/2, mitofusin 1 and 2; OPA1, optic atrophy 1; Parkin, component of a multiprotein E3 ubiquitin ligase complex; PINK1, PTEN-induced putative kinase 1.

mitophagy as an important process that decreases cardiac injury. Paradoxically, excessive mitophagy in response to stress, including I/R, can increase cardiomyocyte death (179). During I/R, MPTP opening leads to depolarization of $m\Delta\Psi$ that is critical to triggering mitophagy by recruiting the E3 ubiquitin ligase Parkin from the cytosol to the mitochondrial surface via the PTEN-induced putative kinase 1 (PINK1) (180, 181). Upon $m\Delta\Psi$ depolarization, PINK1 import and degradation within mitochondria are inhibited; it accumulates on the outer mitochondrial membrane, resulting in recruitment of Parkin to the uncoupled mitochondria (182). Parkin attached to mitochondria ubiquitinates mitochondrial proteins, which are then degraded (8). Parkin can also be recruited to mitochondria through a PINK1-independent mechanism. Ubiquitinated mitochondrial proteins are recognized by the autophagy adaptor protein p62/sequestosome 1 (183); p62 bridges ubiquitinated proteins with LC3 on autophagosomes (184), which engulf the damaged mitochondria.

Genetic deletion of PINK1 and Parkin has shown that these proteins are required for mitophagy in response to I/R (185). Depolarized mitochondria, especially as an index of MPTP,

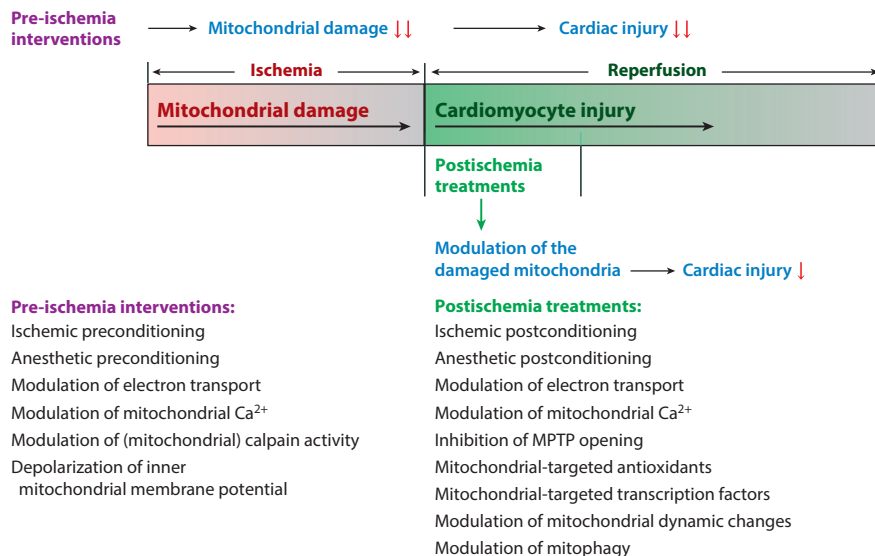


Figure 6

Strategies to decrease mitochondria and myocardial injury during ischemia-reperfusion. Mitochondria, which are damaged progressively during ischemia, set the stage for progressive myocardial injury during reperfusion. Therefore, pre-ischemic interventions, which include ischemic preconditioning (205), anesthetic preconditioning (111), modulation of electron transport (1), modulation of mitochondrial Ca^{2+} (108, 110, 111), modulation of mitochondrial calpain activity (206), and depolarization of inner mitochondrial membrane potential (66, 67), are more effective at reducing cardiac injury during reperfusion in that these interventions protect mitochondria from ischemic damage. Modulation of the ischemic-damaged mitochondria during early reperfusion, which include ischemic postconditioning (207–209), anesthetic postconditioning (134, 135), modulation of electron transport (93, 114), modulation of mitochondrial Ca^{2+} (110, 111), inhibition of mitochondrial permeability transition pore (MPTP) opening (112), mitochondrial-targeted antioxidants (86, 210), mitochondrial-targeted transcription factors (126, 127), modulation of mitochondrial dynamic changes (174), and modulation of mitophagy (187), can attenuate myocardial injury during reperfusion. Treatments during early reperfusion are less effective than interventions before ischemia but have more clinical relevance in that the imminent onset of a myocardial infarction is rarely known.

render the cardiomyocyte at risk for necrotic cell death. PINK1 and Parkin thus provide a branch point for cell survival by enhancing mitophagy, an alternative to necrotic cell death (186). It is unclear if the modulation of mitochondrial metabolism that protects mitochondria at reperfusion is mediated in part by minimizing the need for remodeling of the mitochondria (autophagy, fusion, and fission) so that activation of excessive, damaged mitochondrial remodeling is averted. Attenuation of mitochondrial-driven injury at the onset of reperfusion should lead to mitochondrial fusion, which in turn will decrease the stimulus for activation of mitophagy, resulting in less tissue injury. Therapeutics directed at PINK1 and Parkin are being pursued; the impact of such agents on reperfusion pathophysiology and myocardial salvage will be of great interest. Alternatively, targeting $m\Delta\Psi$ to depolarize and enhance mitophagy is a potential therapeutic avenue (187).

SUMMARY, FUTURE DIRECTIONS, AND THERAPEUTIC IMPLICATIONS

Ischemia leads to a mitochondrial phenotype that favors direct chemical- and signaling-mediated cardiomyocyte injury (Table 1). Ischemia results in the following:

Table 2 Pharmacological interventions to decrease cardiac injury during ischemia-reperfusion

Drugs	Mechanisms of protection	Reference
Pre-ischemia interventions		
Ischemic preconditioning		
Dioxide	K _{ATP} channel opener, complex II inhibitor	136
Nicorandil	K _{ATP} channel opener	137
Anesthetic preconditioning		
Sevoflurane	ROS ↓, intracellular Ca ²⁺ ↓, mitochondrial Ca ²⁺ ↓, complex II inhibitor	111
Modulation of electron transport		
Amobarbital	Reversible complex I inhibitor, OXPHOS ↑, ROS ↓, MPTP ↓	22
AP5A	Reversible complex II inhibitor, ROS ↓	60
3-Nitropropionic acid	Reversible complex II inhibitor	65
Ranolazine	Complex I inhibitor, ROS ↓, intracellular Ca ²⁺ ↓, mitochondrial Ca ²⁺ ↓	97
Mito-SNO	Complex I inhibitor, ROS ↓	98
3-Mercaptopropionylglycine	Complex I inhibitor, ROS ↓	99
Nitrite	Complex I inhibitor, ROS ↓, MPTP ↓	100
Modulation of mitochondrial Ca ²⁺ content		
Ruthenium red	Mitochondrial Ca ²⁺ uniporter inhibitor, mitochondrial Ca ²⁺ ↓	108, 109
Benzamide	Na ⁺ -H ⁺ exchange 1 inhibitor, intracellular Ca ²⁺ ↓, mitochondrial Ca ²⁺ ↓	110
KB-R7943	Na ⁺ -Ca ²⁺ exchange inhibitor, intracellular Ca ²⁺ ↓, mitochondrial Ca ²⁺ ↓	111
Modulation of cytosolic and mitochondrial calpain activity		
MDL-28170	Inhibition of mitochondrial calpain 1, OXPHOS ↑, MPTP ↓	162, 165
Depolarization of inner mitochondrial membrane potential		
Dinitrophenol	Prevention of reverse flow–induced ROS generation	66, 67
Postischemia treatments		
Ischemic postconditioning		
Dioxide	Activation of RISK pathway, S-nitrosylation of mitochondrial protein	208
Hydrogen sulfide	Activation of the JAK2/STAT3 (SAFE) signaling pathway	209
Anesthetic preconditioning		
Sevoflurane	Intracellular Ca ²⁺ ↓, opening of mitochondrial K _{ATP} channel	134, 135
Modulation of electron transport		
Amobarbital	Reversible complex I inhibitor, OXPHOS ↑, ROS ↓, MPTP ↓	33, 93, 114
Metformin	Complex I inhibitor, AMPK ↑, MPTP ↓	32
Modulation of mitochondrial Ca ²⁺ content		
Benzamide	Na ⁺ -H ⁺ exchange 1 inhibitor, intracellular Ca ²⁺ ↓, mitochondrial Ca ²⁺ ↓	110
Inhibition of MPTP opening		
Cyclosporin A	Inhibition of MPTP opening	112, 113
Mitochondrial-targeted antioxidant		
SS-31	Cardiolipin content ↑, OXPHOS ↑, mitochondrial cytochrome <i>c</i> ↓	86
MitoQ	ROS ↓, MPTP ↓	210

(Continued)

Table 2 (Continued)

Drugs	Mechanisms of protection	Reference
Mitochondrial-targeted transcription factors		
Oncostatin M	Activation of mitochondrial STAT3	127
Leptin	Activation of mitochondrial STAT3	126
Modulation of mitochondrial dynamic change		
Mdivi-1	Inhibition of mitochondrial fission	176
TATp110	Inhibition of mitochondrial fission	176
Modulation of mitophagy		
Chloroquine	Mitochondrial uncoupling, induction of autophagy	187

Abbreviations: AMPK, AMP kinase; MPTP, mitochondrial permeability transition pore; OXPHOS, oxidative phosphorylation; ROS, reactive oxygen species.

1. Damage to the ETC that generates oxidant production, leading to impairment of mitochondrial components, including the chain itself (11).
2. Outer mitochondrial membrane changes that favor activation of programmed cell death (188), a phenotype that is protected partially by inhibition of ETC-driven mitochondrial injury.
3. Activation of calcium-mediated and oxidant-generated mitochondrial signaling that includes the pathological onset of MPTP and activation of mitochondria-localized calpains (189).
4. Formation and activation of mitochondria-based cell death signaling that includes formation of cytochrome *c* cardiolipin peroxidase and possible modification of mitochondrial peptides that promote MPTP activation (190).

These multiple mitochondrial responses signal to the cardiomyocyte that a critical portion of its mitochondria are injured. The cellular response involves activation of repair and compensatory systems at the transcriptional (191), posttranscriptional (192), and posttranslational levels (193, 194). Thus, ETC-dependent, ischemia-induced processes persist for prolonged periods of reperfusion. Blockade of electron transport induces protective changes in outer mitochondrial membrane proapoptotic peptides (95) and susceptibility to MPTP (64, 95), thus implying that ischemic alterations in nonoxidative mitochondrial functions result from damaged ETC. During early and later reperfusion, cardiomyocyte responses to ischemia-damaged mitochondria perpetuate mitochondrial damage well into the reperfusion period.

Recognition of ETC-driven responses that propagate mitochondrial-driven pathology in the myocyte suggests interventions that might attenuate the sequelae of ischemic injury during reperfusion. Cellular responses to the presence of ischemia-damaged mitochondria could be addressed during reperfusion to reach back into ischemia to avert the consequences of mitochondrial damage during reperfusion. Because mitochondria are major players in myocardial ischemic injury, there are several types of possible intervention, including the activation of mitophagy (8, 195) and of mitochondrial biogenesis (196, 197) by the use of a variety of currently available pharmacological agents. Also helpful might be interventions that modify function by posttranslational modification of mitochondrial proteins (198, 199) or modulation of transcription factors that regulate mitochondrial metabolism (118). Conceptual approaches to attenuate mitochondria-driven cardiomyocyte injury during ischemia as well as during reperfusion are summarized in **Figure 6**. Potential pharmacologic approaches that address these mechanisms of mitochondria-driven injury are listed in **Table 2**.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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