Review

CARDIOPROTECTION BY PRE- AND POST-CONDITIONING: IMPLICATIONS FOR THE ROLE OF MITOCHONDRIA

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Abstract

The mitochondrion has evolved as an important organelle in determining cell survival and cell death. It is involved in a plethora of processes in mammalian cells including ATP production, steroid synthesis, and cell division and cell death. Indeed, mitochondrial dysfunction is associated with numerous human maladies including heart disease. Mitochondrial diseases have traditionally been attributed to defects in the electron transport chain (ETC), the major source of mitochondrial reactive oxygen species (ROS), a byproduct of mitochondrial respiration. Mitochondrial cation channels and exchangers function to maintain matrix homeostasis and are likely involved in modulating mitochondrial function in part by regulating $O_2^{\cdot-}$ generation. Insofar as mitochondria are involved in oxidative damage that leads to apoptosis, antioxidants and other therapeutic strategies that target the organelle appear to be a novel approach to alleviate some cardiovascular diseases. This novel approach has gained unprecedented attention recently with a significant potential for future therapeutic purpose. Whether mitochondria are targets or end effectors of cardiac pre- and post-conditioning remain unresolved. This brief review will provide the latest information gleaned from the literature on the role of mitochondria in pre- and post-conditioning during cardiac ischemia and reperfusion.

List of frequently used abbreviations:

ROS, reactive oxygen species
RNS, reactive nitrogen species
NADH, nicotinamide adenine dinucleotide (reduced)
$O_2^{\cdot-}$, superoxide anion radical
$NO^{\cdot}$, nitric oxide radical
GSH, glutathione (reduced)
ETC, electron transport chain
DY_m, mitochondrial transmembrane potential
IMM, inner mitochondrial membrane
OMM, outer mitochondrial membrane
IMS, intermembrane space
H_2O_2, hydrogen peroxide
$ONOO^-$, peroxynitrite
SOD, superoxide dismutase
Q, coenzyme Q_{10}, ubiquinone, quinone
$FADH_2$, flavin adenine dinucleotide (reduced)
mPTP, mitochondrial permeability transition pore
VDAC, voltage dependent anion channel
HKI and HKII, hexokinase I and II
MnSOD, manganese superoxide dismutase
MnTBAP, Mn(II)tetrakis(4-benzoate) porphyrin chlorine
OXPHOS, oxidative phosphorylation
UCP, uncoupling proteins
ANT, adenine nucleotide translocase
NHE, Na^+/H^+ exchange
NCE, Na^+/Ca^{2+} exchange
K_{ATP}, ATP-sensitive K^+ channel
K_{Ca}, Ca^{2+} sensitive K^+ channel
IPC, ischemic preconditioning
PPC, pharmacologic preconditioning
APC, anesthetic preconditioning
5-HD, 5-hydroxydecanoic acid
L-NAME, N^G-nitro-L-arginine methyl ester
SNO-MPG, S-nitro-2 mercaptopropionyl glycine
SS, Sezto-Schiller tetrapeptides
IPoC, ischemic post-conditioning
PPoC, pharmacological post-conditioning
APoC, anesthetic post-conditioning
PoC, post-conditioning
GR, glutathione reductase
GPx, glutathione peroxidase
MAPK, mitogen-activated protein kinase

Introduction

Mitochondria are known for their essential role as energy transducers, but mitochondria also perform critical functions such as cell division and apoptosis (59). Mitochondria are effectors of both ischemia and reperfusion (I/R) injury and cytoprotection. Therapeutic targeting of mitochondria has emerged recently as a subject of interest because of the plausible alternative approach this offers in our efforts to treat some diseases. The implication or association of mitochondria in the etiology of many disease states, including heart disease, has heralded novel therapeutic approaches aimed to specifically target mitochondria.

Overall cellular function is dependent on O_2

consumption by functioning mitochondria to produce energy with minimal electron leak to generate the superoxide ($O_2^{\bullet-}$) anion (15,71). Mitochondria are therefore vital for normal cellular function including intracellular metabolic activities and signal transduction of various cellular pathways during normal and abnormal states. Cell signaling pathways induced physiologically by reactive oxygen species (ROS) include effects on thiol groups and disulfide linkages, which post-translationally modifies protein structure to activate/inactivate specific kinase/phosphatase pathways (15). Similarly, mitochondrial proteins can be targets of extra-matrix signaling molecules with concomitant changes in mitochondrial bioenergetics and cellular protection against oxidative stress. Mitochondrial constituents such as proteins, lipids, and mitochondrial DNA are themselves targets of oxidative/nitrosative stress, and the mitochondrial control of oxidative stress has consequences both for cellular energy metabolism and for the processes that control the onset and progression of the cell death response (15). A protected electron transport chain (ETC) during pre- and post-conditioning is therefore critically important for the recovery of the post-ischemic heart.

An understanding of mitochondrial function in normal and pathological states is essential to ameliorate or prevent mitochondria related cardiac dysfunction, like I/R injury. Thus, the recent spotlight on mitochondria is attributed to its role in cell death, in which excess ROS ($O_2^{\bullet-}$ and its products) and dysfunction in the energy production process are underlying factors. $O_2^{\bullet-}$, which is generated at several sites within the ETC and the matrix, is mostly converted to H_2O_2 both inside and outside the mitochondrial matrix by $O_2^{\bullet-}$ dismutases. H_2O_2 is a major, highly diffusible, chemical messenger that, in low amounts, physiologically modulates cell function (15).

Diseases of the mitochondria appear to cause most damage to cells that are metabolically active, like the heart, brain, liver, kidneys and skeletal muscle. In a recent comprehensive review (15), we discussed several mitochondrial related diseases, which will not be discussed here. The objective of this brief review is to provide the role of mitochondria in I/R injury and to note the potential therapeutic approaches to mitigate mitochondria-related dysfunction, specifically pre- and post-conditioning.

Overview of Mitochondrial Anatomy and Function: Implications for Ischemia and Reperfusion Injury and Cytoprotection

Mitochondria are the key cellular organelles charged with synthesizing ATP via oxidative phosphorylation (OXPHOS) (Figure 1). They have major roles in cellular physiology beyond ETC and OXPHOS. Mitochondria are also involved in intracellular Ca^{2+} homeostasis, ROS production, and apoptosis. Mitochondrial compartments include the outer and inner mitochondrial membrane (OMM and IMM, respectively) (Figure 1), the inter-membrane space (IMS), and the matrix. The membranes and the IMS are intimately involved in control of cell function and cell death.

The OMM plays an important role in controlling mitochondrial activity. The OMM and IMS limit the passage of proteins and small solutes that regulate many cellular processes and initiate or inhibit apoptotic pathways. The OMM is a relatively simple phospholipid structure that contains many channel proteins. OMM permeabilization is considered the 'point of no return' as this event is responsible for initiating the apoptotic cascade in numerous cell death pathways (22). The release of, for example, cytochrome c triggers formation of an "apoptosome" that leads to activation of the executioner caspases (22). It is possible that under normal conditions changes in mitochondrial dynamics also modulate OMM permeabilization to induce apoptosis (17,18).

The most abundant protein on the OMM is the VDAC. It acts as a conduit for translocating ions and a variety of metabolites such as ATP and ADP across the OMM. As a major gateway in and out of the mitochondrion, VDAC mediates a delicate balance between metabolism and death in cells (15). However, following I/R, a 4-fold increase in VDAC phosphorylation has been reported (66) and the cardioprotective effect of PD169316, an antagonist of p38 mitogen-activated protein kinase (MAPK) resulted in a significant reduction in ischemia-induced phosphorylation of VDAC, specifically a reduction of tyrosine phosphorylation (66).

The VDAC is also a receptor for hexokinases (HK) and the VDAC-HK binding has been implicated in cell survival and cell death. HK II binding to VDAC is believed to maintain mitochondrial membrane integrity and to prevent the Ca^{2+}-dependent opening of the mitochondrial permeability transition pore (mPTP) (80). The mPTP is a mega-pore that connects IMM and OMM proteins with the result that mitochondrial permeability increases and cell demise ensues. Exposure to apoptotic stimuli causes a decline in mitochondrial HK II activity (31). In the heart, it has been suggested that the mitochondria-HK interaction may indeed be an integral part of cardioprotection, including ischemic and pharmacologic preconditioning (IPC and PPC, respectively) (85).

In addition to mCa^{2+}, mPTP opening appears to be instigated by high levels of ROS, increased P_i and a high matrix pH (15). Since matrix pH likely becomes more acidic during ischemia, pore opening will not be favored. Therefore, matrix acidification during I/R has

been shown to be a beneficial strategy in attenuating I/R damage (15). In contrast, matrix alkalinization on reperfusion may induce pore activation, so continued acidification during reperfusion or inhibition of Na^{2+}/Ca^{2+}-exchanger (NCE) to minimize cytosolic and mitochondrial Ca^{2+} overload would be strategically beneficial during this period to reduce cell death. Indeed, acidification has been shown to be effective in post-conditioning protection as demonstrated by inhibited pore opening (15).

We and other investigators have also assessed myocardial mPTP opening in the intact heart, prior to the onset of contractile dysfunction during I/R, and in isolated mitochondria (2,10,33). We showed that I/R increases the vulnerability of isolated mitochondria to increased extra-matrix Ca^{2+} challenges (2). In the cardiac dystrophied mdx mouse model, evaluation of mPTP in the intact isolated heart model using the ^{3}H-DOG method showed increased mPTP opening as evidenced by release of cytochrome c when hearts underwent I/R (10). Mitochondria from isolated hypertrophied rat hearts exhibited an increased susceptibility to opening of mPTP in response to physiological stressors (i.e., Ca^{2+} overload and anoxia and reoxygenation) and H_2O_2 (10,15).

In the IMM, a large DY_m (negative inside) is formed across the IMM by outward proton pumping by the respiratory enzymes of the ETC. The IMM charge separation and transmembrane cation fluxes via specialized cation channels/uniporters, symporters, and exchangers are essential for mitochondrial respiration and function (15). Activation of the exchangers allows extrusion of cations entering the matrix down their concentration gradient, thus preventing volume expansion. The IMM also contains the adenine nucleotide translocator (ANT), which is considered a crucial component of the mPTP. The ANT has been shown to be a target for oxidative and nitrosative stress and therefore is an important component in I/R injury. In cardiac myocytes, connexin 43 (Cx43), the predominant protein in gap junctions in ventricular myocardium, is also localized in the IMM of cardiomyocyte mitochondria (15) and has been implicated recently as a factor in pre- and post-conditioning (65).

There is increasing evidence that mitochondria, which have a large capacity to take up Ca^{2+}, play a critical role in maintaining intracellular Ca^{2+} homeostasis in I/R (15). During I/R, increased cytosolic Ca^{2+} may lead to mCa^{2+} overload via the mitochondrial Ca^{2+} uniporter (Figure 1), and in the presence of ROS, may lead to mPTP opening and cell death. Therefore, preventing Ca^{2+} uptake at times of increased cytosolic Ca^{2+} due to oxidative stress has proven to be a viable strategy to mitigate cellular damage during I/R. In a recent study it was reported that melatonin, a pineal gland hormone protect against brain I/R injury in part by preventing

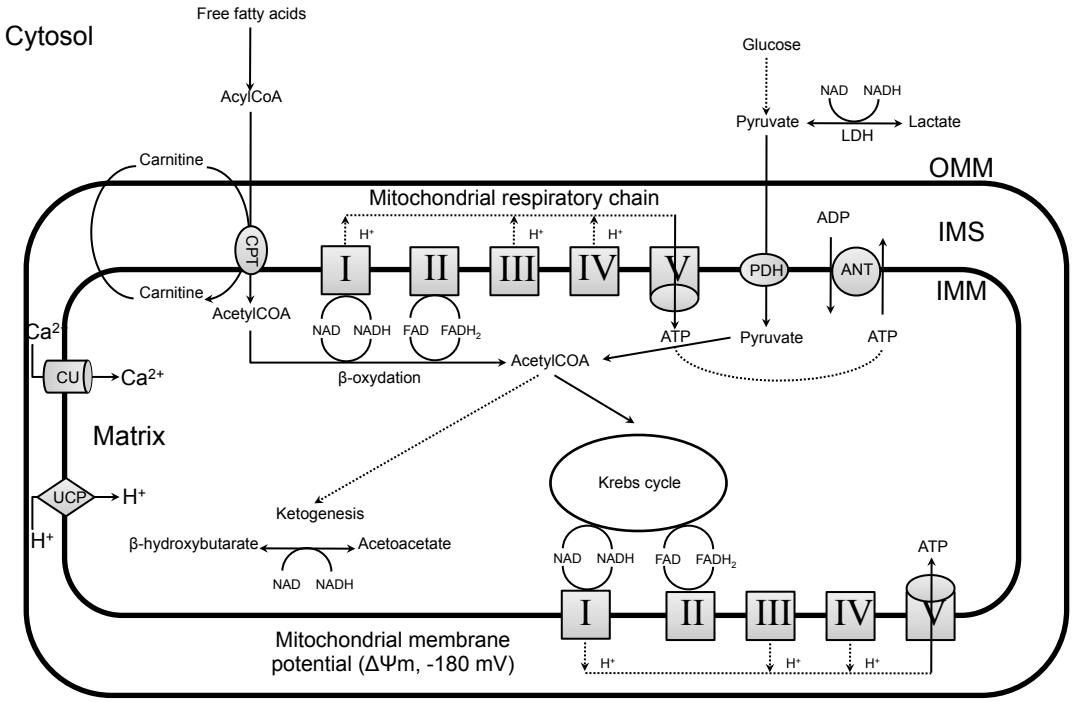

Figure 1: Figure shows basic structural components of the ETC complexes as well as the sites of proton pumping during electron transport that leads to the generation of ATP. NADH and $FADH_2$ (from Krebs cycle) needed to energize mitochondria and establish the mitochondrial membrane potential (ΔY_m; -180 to -200 mV). The ΔY_m can be modulated be uncoupling proteins (UCP). Phosphates are imported and exported through the adenine nucleotide translocase (ANT). Substrate uptake is mediated through mitochondrial inner membrane (IMM) proteins [e.g., carnitine palmitoyl transferase (CPT) and pyruvate dehydrogenase (PDH). Ca^{2+}, which may modulate mitochondrial respiration, is taken up via the calcium uniporter (CaU).

mCa^{2+} mediated apoptosis (35).

In mitochondria K^+ flux also plays a significant role in normal mitochondrial function and cytoprotection against apoptosis. The putative mitochondrial K_{ATP} (mK_{ATP}) and the mitochondrial K_{Ca} (mK_{Ca}) channels are involved in protection against I/R injury. The mK_{ATP} channel agonist diazoxide has been shown to protect cells from I/R damage in part by minimizing mCa^{2+} uptake through depolarization of DY_m (28,53). A prototype of mK_{ATP} channel opener, BMS-180448, has cardioprotective effects and is currently in clinical trials (58). We recently provided evidence for a regulatory role of the putative mK_{Ca} channel opening on matrix pH and volume (4). We also showed that pre-ischemic exposure to the big K_{Ca} channel agonist, NS1619 provided protection against I/R injury by reducing mitochondria-mediated ischemia-induced ROS production (70). However, the role of mitochondrial K^+ channels in cell protection remains uncertain because the molecular structure of these channels has not been identified.

The IMM also contains the ETC complexes that furnish the structures for proton translocation, and in the process function to convey electrons down the ETC along an energy gradient (15,41,71). Electrons pass from NADH and $FADH_2$ via complexes I and II, respectively, to downstream complexes (Fig 1). Translocation of proton along the electrochemical gradient from the IMS to the matrix through complex V (F_1/F_0 ATPsynthase) drives phosphorylation of ADP to ATP (15,71) and transiently dissipates the DY_m. In the process of electron transport, electrons can leak resulting in $O_2^{\cdot-}$ generation. The leak is more severe during I/R and hence the combining of O_2 and free electrons contributes to the pathology of mitochondria.

We and other investigators have shown that targeting the ETC complexes for therapeutic purpose is in part attributed to the vulnerability of mitochondria to oxidative stress. We observed and reported that the partial complex I blocker amobarbital protected against I/R injury when the drug was perfused briefly only just before ischemia; we concluded from this study that the protection was mediated by limiting electron flow from upstream to complex III, a major source of ROS (3,15). These observations are consistent with studies that show rotenone, a complex I blocker given on reperfusion, did not provide as much protection in preserving the integrity of mitochondrial structure and function, as when the drug was administered before ischemia (20). Therefore, maintaining the structural and functional integrity of mitochondrial complexes and associated structures is a prerequisite for normal cell function. Furthermore, these studies highlight an emerging paradigm that reversible metabolic inhibition may be a common pathway leading to cellular protection and that the ETC regulates apoptosis (15).

The uncoupling proteins (UCPs) are IMM transporters that control the rate of electron transfer through the ETC (62). Mitochondrial uncoupling is an important physiological regulator of mitochondrial function because of its effect on redox potential and $O_2^{\cdot}$ production (15). A small class of "uncoupling" proteins called UCPs 1-4 and fatty acids are believed to induce an inward proton "leak" in charged mitochondria (15,71). Uncoupling agents have shown promise as potential mediators of cytoprotection by minimizing the levels of cytotoxic ROS. For instance, activation of the putative mK_{ATP} channels may lead to mild uncoupling of mitochondrial respiration via mild "proton leak" with a concomitant increase in $O_2^{\cdot-}$ generation that provides the signal for protection against ischemic damage (15); and recent studies have shown mild uncoupling of

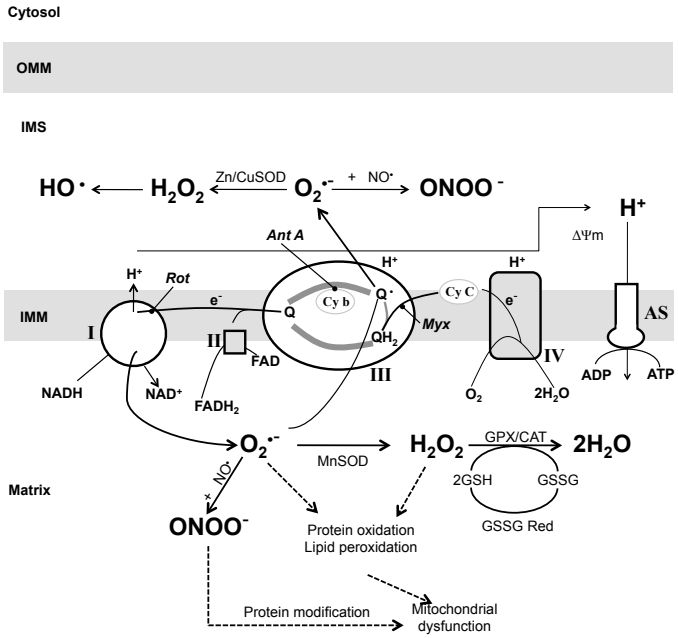

Figure 2: Principal mechanisms for mitochondrial ROS and RNS generation. Reduced substrates synthesized in metabolic pathways supply electrons (e^-) to ETC complexes. The major centers for $O_2^{\cdot-}$ formation are complexes I and III. The electron carrier of complex III ubiquinone (Q) is reduced to ubiquinol (QH_2), which transfers an electron to cytochrome c (Cy C) through an Fe protein (not shown) inhibited by myxothiazol (Myx). The resulting semiubiquinone ($Q^{\cdot}$) is oxidized back to ubiquinone by cytochrome b (Cy b), and can also transfer e- to O_2 to form $O_2^{\cdot-}$. Myxothiazol reduces $O_2^{\cdot-}$ production because it blocks $Q^{\cdot}$ formation, whereas antimycin A (Ant A) enhances it by increasing $Q^{\cdot}$ levels. The drug rotenone (Rot) inhibits complex I to generate some $O_2^{\cdot-}$. The $O_2^{\cdot-}$ generated by Myx are directed to the matrix where they are scavenged; $O_2^{\cdot-}$ generated by Ant A are released into the IMS and so escape the powerful matrix scavengers. The figure also depicts the dismutation of $O_2^{\cdot-}$ and its reaction with $NO^{\cdot}$ to form $ONOO^-$. Reproduced with permission Camello-Almaraz et al (16). SOD, superoxide dismutase; H_2O_2, hydrogen peroxide; $ONOO^-$, peroxynitrite; $NO^{\cdot}$, nitric oxide; DY_m: mitochondrial potential; AS, ATPsynthase; OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; IMS intermembrane space, CAT catalase, reduced GSH glutathione, oxidize glutathione GSSG.

DY_m may be a mechanism of anesthetic preconditioning (APC) (67). These interesting findings could provide new insight as to the mechanisms by which anesthetic preconditioning confers protection against I/R injury.

Mitochondrial Reactive Oxygen and Nitrogen Species: Role in Cell Survival and Injury

Reactive oxygen species, including superoxide anions ($O_2^{\cdot-}$) hydrogen peroxide (H_2O_2), and ferryl hydroxyl radicals ($FeOH^{\cdot}$) are continuous byproducts of normal mitochondrial aerobic metabolism (Figure 2) that can oxidize nucleic acids, lipids and proteins, and in other cases nitrosylate proteins leading to the modification and/or loss of functions. Under normal conditions, potentially toxic ROS generated by mitochondria ETC are efficiently detoxified by mitochondrial and cytosolic scavenging systems. However this balance can be upset and lead to excess ROS and reactive nitrogen species (RNS), like nitric oxide ($NO^{\cdot}$) and peroxynitrite ($ONOO^-$) (15). Therefore, the balance between ROS and RNS generation and their detoxification is a critical determinant of cell survival and cell death. To maintain this delicate balance mitochondria are equipped with endogenous antioxidant defenses that regulate $O_2^{\cdot-}$ within a physiological range (Figure 2).

Physiological levels of ROS are important in that they are key part of cell signaling molecules for normal cell function as well as in cytoprotection during pre- and post-conditioning. Under pathological conditions, the delicate balance (generation – scavenging) that keeps the level of $O_2^{\cdot-}$ to a minimum is altered so that the rate of $O_2^{\cdot-}$ generation exceeds the rate of scavenging. By maintaining the level of antioxidants in the matrix and by minimizing electron flow to complex III, we may have a potential therapeutic approach to neutralize oxidative-stress-induced mitochondrial dysfunction and subsequent apoptosis.

Tissue damage during disease processes has long been known to be associated with increased levels of ROS. Increased mitochondrial ROS production, for example during cardiac I/R injury, can impair complex I (46), which in turn can enhance $O_2^{\cdot-}$ formation as a result of increased electron leak as electron transfer is impeded (15). Studies have shown that an increase in H_2O_2 generation in mitochondria isolated after cardiac I/R injury was enhanced by either rotenone or antimycin A; this suggested that both complexes I (Q and/or N2 (final) Fe-S center) and III were damaged and capable of producing $O_2^{\cdot-}$ after I/R injury (Figure 2) (15,71).

In addition to induction of ROS by hypoxia, ischemia, and mitochondrial toxins/drugs, ROS per se may lead to even greater ROS generation in a self-amplifying manner. This phenomenon, called ROS-induced ROS release, may be associated with mCa^{2+} overload and may play a role in initiating apoptosis; but whether normal stimuli initiate it is not known (71). It seems unlikely that Ca^{2+} overload can itself induce ROS generation because it dissipates DY_m and lowers the redox state (16); but it may hinder the ROS scavenger system so that more ROS is produced (15,71). In our studies though, during early ischemia in isolated hearts, ROS increased along with mCa^{2+} and redox state (NADH) (3,5,13) and treatment with exogenous ROS scavengers reduced ROS and mCa^{2+} and better maintained redox state (NADH) during I/R injury (13,70).

The RNS $NO^{\cdot}$ and its derivatives are mediators of numerous vital physiological processes including cytoprotection; they are also mediators of cell injury (69). $NO^{\cdot}$ binds to the O_2 binding site of complex IV, blocking energy production in the same way as hypoxia. This may be one way in which cells regulate their energy production. It may also be one way in which our body kills pathogens. Physiologically, $NO^{\cdot}$ has four major effects on mitochondria: 1) inhibition of complex IV, 2) inhibition of complex I, 3) indirect stimulation of mitochondrial biogenesis, and 4) pathologically full activation of mPTP. These actions of $NO^{\cdot}$ can be direct, such as binding to complex IV or mPTP activation, or through $NO^{\cdot}$ derivatives. $NO^{\cdot}$ generates $ONOO^-$ when it reacts with mitochondrial $O_2^{\cdot-}$; $ONOO^-$ at high concentrations activates opening of the mPTP. Other $NO^{\cdot}$ based products, such as S-nitrosothiols (SNO), inhibits complex I, which in turn can increase $O_2^{\cdot-}$ and H_2O_2. SNO can also activate mPTP opening (15).

At low concentrations $NO^{\cdot}$ provides cytoprotection from oxidative stress induced by I/R injury or hypoxia with reoxygenation. Inhibition of complex IV by $NO^{\cdot}$ is thought to elicit cardioprotection by protecting the limited supply of O_2. Other experimental evidence to date supports the cytoprotective effect of $NO^{\cdot}$ binding to complex IV as shown by resistance to apoptosis (24). $NO^{\cdot}$ is strongly implicated in the mechanisms underlying cytoprotection mediated by ischemic preconditioning (IPC) in part by its inhibition of complex I (15,24). SNO inactivation of complex I results in inhibition of respiration and causes a small increase in $O_2^{\cdot-}$ and H_2O_2 generation. It is thought that the generation of ROS may be one of the signaling mediators of preconditioning (40,72).

Potential Therapeutic Implications for Mitochondrial ROS Scavenging

ROS and RNS are clearly involved in normal cellular functions, cellular injury, and cytoprotection. In a recent review (15) we asked could "modulation of ROS/RNS be an effective therapeutic tool?" To address this question the need to efficiently neutralize pathologic ROS and RNS has to be balanced with the need to maintain these species at physiological levels. The mitochondria and the cell are both equipped with an

armament of radical scavengers. These include the intra- and extra-matrix SODs, and glutathione (GSH) (71) systems, catalase (cytosolic and possibly mitochondrial) GSH peroxidase and cytochrome c (15,71). Detailed review of ROS scavengers, which is beyond the scope of this review, has been discussed in our recent comprehensive review articles by the authors (15,71).

MnSOD (SOD2) is located primarily in the mitochondrial matrix. It is only known role is to detoxify $O_2^{\cdot-}$ to H_2O_2, thereby protecting the mitochondrial Fe-S cluster containing enzymes from oxidative damage (15). Mitochondrial SOD mimetics (non-protein) have been developed to allow uptake into the mitochondrion to scavenge ROS. This includes, the synthetic metalloporphyrins, manganese (III) tetrakis (4-benzoic acid) porphyrin (MnTBAP). Unlike enzymatic exogenous SODs the metalloporphyrins are highly permeable (15).

We reported that MnTBAP alone abolished cardioprotection by mK_{Ca} channel opening (70) and IPC (37), suggesting the importance of ROS in the mechanism of cytoprotection in these studies. The metalloporphyrins have also gained attention recently in organ and tissue transplant. Studies have shown that oxidative stress resulting from I/R can cause endothelial dysfunction in the vein graft with the possibility of graft failure. The addition of MnTBAP during coronary and lower limb arterial reconstruction improved endothelium-dependent vasorelaxation in harvested saphenous vein (68). Attempts to incorporate these therapies in the realm of human organ transplantation, however, remain unresolved. One would hope in time that with increased oxidative stress due to reduction in endogenous scavengers in allografts (64), the potential beneficial role of synthetic MnSOD mimetics would become clinically applicable.

Glutathione is a tri-peptide with the thiol (-SH) residue of cysteine as its active site; it is regulated by the cytosolic redox state. It provides protection for mitochondria against endogenous ROS. The high reducing power of GSH also makes it a major contributor to the recycling of other oxidants that have become oxidized and could be a basis by which GSH helps conserve lipid-phase antioxidants like a-tocopherol (vitamin E) (48,49). Mitochondria appear to be the most susceptible target in the GSH-depleted state. GSH protects mitochondria from lipid peroxidation by reducing phospholipid hydroperoxides and H_2O_2 (7).

Catalase has been found in cardiac mitochondria, albeit in minute amounts. It protects the organelle against intra- and extra-mitochondrial generated H_2O_2 (7). Glutathione peroxidase (GPx) is ubiquitously expressed in mammalian tissues and is present in the mitochondrial matrix. Because of its quantity, it seems to be the predominant H_2O_2 detoxifying agent in the heart. Catalase or GPx coupled to glutathione reductase (GR) converts H_2O_2 to H_2O (Figure 2). There are variant isoforms of GPx. While some of the variants are involved in detoxifying H_2O_2, other isoforms are involved in neutralizing lipid hydroperoxides (30) (Figure 2).

Lastly, cytochrome c by its nature is a scavenger. It is an effective electron acceptor and has been reported to act as a competent antioxidant system of mitochondria. It mediates and relays electron transfer between two sites that are critical in the generation of $O_2^{\cdot-}$, the site of electron transfer to complex IV and generation of $O_2^{\cdot-}$ in complex III. Thus it is also a scavenger of $O_2^{\cdot-}$ through its capacity to be reduced (15).

In cardiac I/R injury, and other acute diseases, loss of cytochrome c inhibits respiration and this can lead to increase electron leak (71); the outcome is more $O_2^{\cdot-}$ production and more cell damage. Protecting cardiolipin from I/R damage could help to preserve cytochrome c and will not only facilitate the transfer of electrons, but also contribute to the scavenging of ROS. In a recent study, Brooks et al. (9) showed that acute kidney damage was associated with increased mitochondrial fragmentation prior to cytochrome c release and apoptosis. Administration of exogenous cytochrome c to cytochrome c-depleted mitochondria reduced $O_2^{\cdot-}$ accumulation and preserved organ function (84). Hence, maintaining the structural integrity of the IMM and cytochrome c could represent a potentially useful strategy for mitigating mitochondria-related cellular injury.

It is often noted that excess ROS and mCa^{2+} overload are the two major factors that are intertwined in the pathology of I/R injury. But how they are interrelated or how they influence each other is a subject of intense debate. It suffices to note here that mCa^{2+} overload could lead to ROS production in part by mPTP opening and loss of GSH and NADH; these are all key factors involved in maintaining the redox balance in the GSH/GSSG system and thus provide efficient scavenging capacity (7) (Figure 2). Therefore, enhancing the endogenous levels of the GSH pool is a potentially viable strategy to protect against mitochondria-related cellular injury. Thus pharmacologic and/or molecular approaches to preserve or minimize GSH depletion could be a useful option for protecting mitochondria. Alternatively, pharmacological or ischemic (pre- and post-conditioning) approaches could be made to instigate protection mediated through a myriad of events that preserve mitochondrial redox state and protect the cell.

Mitochondria and Targeted Therapeutic Approaches

Given the importance of mitochondrial events during the initial, critical phases of apoptosis, the design of mitochondriotropic drugs is considered a promising novel strategy in regulating apoptosis and cell

injury/death. Szewczyk and Wojtczak noted in a recent review (75) that medically applied drugs can be divided into two groups: 1) those that are specifically designed to affect mitochondrial function, and 2) those for which primary targets are other cellular locations and their interactions with mitochondria are secondary. Identification of the mitochondrion as a target of a therapeutic approach may assist in better understanding of mechanisms of action and provide new perspectives for treating diseases, including cardiovascular diseases. The subject of targeted approach to mitochondrial diseases has been discussed extensively in our recent review (15) and therefore will not be discussed further in this review. This review focuses mostly on pre- and post-conditioning strategies targeted to mitochondria. The premise here is that pre- and post-conditioning effects converge at the conceptual point of preserving the integrity of electron transfer to enhance mitochondrial bioenergetics and to minimize the deleterious effects associated with increased mitochondrial ROS and mCa^{2+} overload (15).

Cardiac Mitochondria Protection against Ischemia and Reperfusion Injury: Implications for the Role of Pre- and Post-conditioning

There is ample support for the concept that mitochondrial function can be protected during global ischemia as well as on reperfusion. This has been demonstrated in many studies, including inhibitors of sarcolemmal and mitochondrial Na^+/H^+ exchanger (NHE), hypothermia, and pre- and post-conditioning (ischemic or pharmacological) protocols. The protection is manifested largely as reduced mCa^{2+} uptake and $O_2^{\cdot-}$ generation, improved redox state (NADH/FAD) and cardiac function, blunted mPTP opening, and a reduced infarction (15).

In a recent study (6) we reported that hyperkalemic, depolarizing cardioplegia was protective by a mitochondrial effect in addition to its effect on sparing high-energy phosphates; in the same study we also showed that lidocaine, a hyperpolarizing agent, also protected hearts in part by a direct action on the mitochondria, a concept supported by other studies (19). We found that lidocaine blocked complex I as suggested by an increased NADH without a change in FAD during lidocaine perfusion just before ischemia. This situation was analogous to what we observed with the complex I inhibitor amobarbital (3), which was also protective against cardiac I/R.

It has been a long-held belief that most cellular injury following I/R is initiated by events that happen on reperfusion. However, some of our recent studies and those by others have shown that events leading to I/R injury occur during ischemia and these events may extend to reperfusion to induce additional damage (15). Restriction of oxidative metabolism during early reperfusion using a hypoxic reperfusate attenuated mitochondrial and cardiac damage (20). In renal I/R-induced oxidative stress, repetitive hypoxic preconditioning attenuated cell damage via a HIF-1a-dependent signaling cascade that led to an increase in the anti-apoptotic Bcl-2 protein expression, inhibition of the cytosolic pro-apoptotic Bcl-2 protein expression, and inhibition of mitochondrial cytochrome c release (81). Initial reperfusion with mitochondrial-targeted peptides was reported to prevent myocardial stunning and to significantly improve contractility (73,74). Therefore, there are a variety of strategies that can target mitochondria in an attempt to interrupt the link between ischemic damage to mitochondria and mitochondria-mediated cellular damage during reperfusion. It is becoming more evident that pre- and post-conditioning strategies and the sequelae of events provide protection in part by targeting mitochondria.

Pre-conditioning and post-conditioning have been demonstrated in numerous organs, including kidney, liver, brain and heart I/R (63,83). Some aspects of pre- and post-conditioning and how they modulate mitochondrial function have been discussed above. The earlier references to these two cardioprotective strategies are inevitable considering the broad aspect of the role of mitochondria in cytoprotection. Nevertheless, ischemic pre- and post-conditioning (IPC/IPoC) are cytoprotective measures that requires brief pulses of ischemic stress that confers resistance to a second, usually more severe stress, index ischemia or reperfusion, respectively. Pharmacological pre and post-conditioning (PPC/PPoC) is a cardioprotective mechanism that mimics IPC/IPoC, in that a memory period of protection is created after removal of the stimulus or drug. Both pre- and post-conditioning mediate their protection in part by mitigating mitochondria-dependent cell damage. The protective effect is mediated by intrinsic pathway after the removal of a stimulus (brief ischemia or a drug) during the time before the onset of damaging ischemia or on reperfusion. Thus the stimulus does not directly induce cytoprotection but rather some downstream signaling factors are evoked to provide a lasting protection (memory effect). Because post-conditioning has the advantage that it can be applied after the ischemic insult has occurred (39,79,82), this is therapeutically a more favorable approach than is pre-conditioning.

IPC and PPC of the heart are known to decrease mitochondrial damage from subsequent index ischemia. IPC was identified as an endogenous cytoprotective phenomenon, whereas PPC has the advantage of not requiring brief episodes of ischemia to elicit cellular protection (15). Of interesting clinical relevance, patients who have experienced angina be-

fore myocardial infarction repeatedly exhibit an improved post-infarction prognosis compared with those without antecedent angina. Pre-conditioning-induced ischemic tolerance has been evaluated in humans during angioplasty and before initiation of cardiac bypass (11).

Because pre-conditioning must be applied before an ischemic event to be protective, this limits its usefulness clinically since ischemia and tissue injury are often not predictable and these procedures are seldom instituted early enough to minimize infarction (77). Post-conditioning (ischemic or pharmacological), however, requires a very brief period of secondary I/R or brief exposure to drugs with subsequent washout, during the early reperfusion period. The period of the post-conditioning protection, which must be implemented immediately upon reperfusion, is significant because it marks the critical time at which maximal cell damage might be reduced (15). This period coincides with surges in ROS production, mCa^{2+} overload, and mPTP opening (79,82), or at least initiation of apoptotic mechanisms. Post-conditioning may also protect against I/R injury at least in part by maintaining an acidic pH during reperfusion, which may inhibit mPTP opening. Delayed opening of the mPTP has also been implicated in PPC afforded cardioprotection. Treatment with atractyloside, an mPTP opener, abolished the infarct-reducing effects of nitric oxide-induced preconditioning (27). Interventions aimed at limiting cell injury during reperfusion i.e. post-conditioning, whether ischemic or pharmacologic, may also be mediated through similar mechanisms. Indeed pre- and post-conditioning have been shown to evoke similar cytoprotective mechanisms/pathways that initiate or end at the mitochondria (15).

The cellular and mitochondrial protection elicited from both IPC and PPC involve a coordinated interplay of multiple trigger and effector mechanisms, mostly phosphorylation and dephosphorylation of select enzymes. There is much circumstantial evidence that mitochondria-derived ROS play an important role in initiating IPC and PPC (15), which are effected by intracellular protein kinase cascades, especially PKCe (36), tyrosine kinases, and MAPK (83). These signaling pathways have been discussed extensively in a review by Yellon and Downey (83), and will not be discussed here. Nonetheless, the key effector signaling pathways for both cytoprotective strategies appear to result in the modulation of mitochondrial oxidative metabolism before prolonged ischemia, or to prevent mPTP opening on reperfusion.

Early experiments showed that IPC in the heart was abrogated when the pre-conditioning pulses were preceded by 5-hydroxy decanoate (5-HD) and not by HMR1098, the selective antagonist for the sarcolemmal K_{ATP} channel. This suggested that the mK_{ATP} channel is a mediator of IPC by modulating mitochondrial bioenergetics. However, prolonged opening of the purported mitochondrial channel during ischemia did not confer protection. It is presently disputed whether 5-HD or glibenclamide, applied after preconditioning just before index ischemia, abrogates protection. But, several studies have shown that the application of 5-HD after the preconditioning protocol abrogated protection (54).

Conversely, administration of diazoxide, the putative mK_{ATP} agonist conferred protection, possibly by inducing a mild depolarization of the DY_m. This would dissipate the energy that is normally used by the F_1F_0 ATPase to produce ATP; hence, mK_{ATP} channel opening would result in mild uncoupling of mitochondrial ATP production (54) to protect the organ. By depolarizing mitochondria, diazoxide may reduce mCa^{2+} uptake and decrease pathological ROS generation, while reducing permeabilization of OMM due to translocation of proapoptotic proteins from the cytosol to the mitochondria or induction of mPTP opening (54). Interestingly, the initial mild uncoupling induced directly by diazoxide may be due to its attenuation of complex II (34); this may lead to a small release of mitochondrial ROS that many believe is the primary initial signaling event that lead to the activation of down-stream end-effectors. Most studies have demonstrated that ROS scavenging prevents IPC, thus implicating the initial requirement for ROS in IPC and PPC. For example the mitochondrial $O_2^{\cdot-}$ scavenger N-mercaptopropionyl glycine was shown to block the protective effects of diazoxide (57).

Much of the work on PPC has centered on the volatile anesthetics and their potential for organ protection during the stress of surgery. The volatile anesthetics have long been known to provide overall protection against the effects of cardiac I/R because of their mild coronary vasodilatory and their negative inotropic effects. These effects correspondingly decrease metabolic demand and increase O_2 supply, while having a direct suppressive effect on myocardial Ca^{2+} entry through L-type Ca^{2+} channels (14). In contrast, protection against cell injury after only a brief exposure to a volatile anesthetic, anesthetic pre-conditioning (APC), provides protection against I/R injury similar to IPC and other drugs, invoking similar signaling pathways and end effectors. As in IPC, scavengers of ROS abrogate APC, suggesting an effect of anesthetic agents to also induce ROS formation (15). The mechanism by which these drugs induce ROS formation is unclear. However, volatile anesthetics directly inhibit mitochondrial ETC complex activity (especially complex I) and thus alter mitochondrial bioenergetics; this strongly implicates the mitochondrion as the target for these effects. Furthermore, the release of small amount of ROS during

APC causes a dramatic decrease in mitochondrial ROS formation during I/R and this might underlie the improved post-ischemic organ function and reduction in infarct size (15).

APC holds an obvious advantage over IPC from the practical standpoint that volatile anesthetics can be easily and safely administered. Novalija et al. (52) reported that APC was manifested by improved cardiac function and decreased infarct size compared to controls. When a mixture of ROS scavengers, i.e. SOD, catalase and GSH, or the NOS inhibitor L-NAME, were given during the sevoflurane pretreatment phase, protection was abrogated in all respects. Kevin et al. (38) similarly pre-conditioned guinea pig isolated hearts with sevoflurane and MnTBAP abrogated the APC. Sedlic et al. (67) reported recently that isoflurane, like DNP, protected cardiomyocytes in part via a mild decrease in Y_m, which attenuates pathologic ROS production under stress and delays mPTP opening and increases cell survival. These studies all strongly support the probability that modulation of mitochondrial bioenergetics by preconditioning induces cytoprotective mechanisms manifested at the level of the mitochondrion (15).

Like the putative mK_{ATP} channel, we have shown that the putative mCa^{2+}-dependent K^+ channel (mK_{Ca}) may also play a role in modulating mitochondrial bioenergetics and provide pre-conditioning protection against cardiac I/R injury (15). We reported that pre-treatment with NS1619, the K_{Ca} channel agonist protected the heart from ischemic injury (70). NS1619, given before ischemia, preserved the mitochondrial redox state (increased NADH and decreased FAD) and improved cardiac function. Paxilline, an NS1619 antagonist, and MnTBAP, both abolished preservation of mitochondrial bioenergetics and cardiac protection by NS1619 (70). These findings attest to the role mitochondria play in the protection and damage of the cell during I/R stress.

It is widely acknowledged that at the cellular level, reperfusion activates a number of processes that increase cell injury. The surge in ROS during late ischemia coupled with the rapid increase in O_2 on early reperfusion contributes to events that lead to a vicious cycle of more ROS/RNS production and further mitochondrial damage. Reperfusion also contributes to an increase in mCa^{2+} overload, which along with increases in ROS/RNS and a relatively less acidic intracellular pH may lead mPTP opening. This results in mitochondrial swelling and uncoupling of the ETC and collapse of the DY_m, cytochrome c release, decline in ATP production, and cell death.

Like IPC/PPC, PoC, ischemic (IPoC) or pharmacological (PPoC), have been shown to be protective in numerous animal models and recent evidence suggests that it may work in humans (51). Administration of a wide variety of drugs (32,47,55,56), immediately on reperfusion has been shown to provide protection that is as effective as IPC. Many of the signaling pathways instigated by pre-conditioning are also implicated in PoC (55). Post-conditioning protection is also mediated in part by targeting the mitochondrion, either as an initial triggering phase of the process, or as an end-effector, or both. For example, in a recent study, Ge et al. reported that the cardioprotection by anesthetic post-conditioning (APoC) appears to be mediated via the mPTP (29). In this study Ge et al. (29) showed that isoflurane post-conditioning failed to alter infarct size, cardiac function, or the amount of Ca^{2+} necessary to activate the mPTP from the $eNOS^{-/-}$ hearts compared with the wild type. It was concluded that APoC protected mouse hearts from reperfusion injury by preventing mPTP opening in an e-NOS dependent manner, and that $NO^{\cdot}$ may be acting as both trigger and a mediator of APoC protection (29).

To date, it is unresolved if similar signaling pathways in pre-conditioning protection are involved in mediating post-conditioning protection. However, from recent evidence it appears that similar signaling mediators, which are activated at the time of reperfusion PoC (55), modulate cytoprotection. In addition, Feng et al. (25) showed that both IPC and PPoC might provide protection by reversing ischemia-induced ANT dephosphorylation and thereby improve ATP production. In a related study, Cheng et al. (21) reported that dephosphorylation of ANT results in an increase in ANT activity following incorporation of the purified protein in a lipid bilayer model. Thus, a dephosphorylated ANT can potentially lead to the detrimental consequence of mPTP opening (21) with subsequent cell injury. Unlike IPC, the major limitation with PoC is that it is unlikely to evoke pro-survival signaling pathways rapidly enough to avert cellular injury early on reperfusion. Therefore other faster remedies independent of signaling proteins could be considered as more effective during the initial phase of reperfusion.

Indeed, in a recent study, Pravdic et al. (60) reported that APoC better preserved matrix acidic pH during reperfusion than the control group, which delayed mPTP opening and contributed to the preservation of mitochondria and better cytoprotection. Similarly, we have observed that post-ischemic hypothermic treatment also protects against I/R damage by protecting mitochondria. We also observed that addition of ROS scavengers during hypothermic post-ischemic perfusion provided additional protection of mitochondria and improved global cardiac function (unpublished data). These novel observations imply that cytoprotective strategies can be elicited by directly targeting mitochondria independent of activation of signaling cascades that require time to be activated. This strat-

egy could herald an alternative approach to mitigate reperfusion-mediated injury.

In another post-ischemic study, we observed that brief perfusion with NS 1619 (within 10 min reperfusion) after 30 min of global ischemia also preserved mitochondria, improved cardiac function, and reduced infarction when compared to untreated hearts. Interestingly, we also observed that brief perfusion of paxilline on reperfusion worsened mitochondrial bioenergetics and further impaired functional recovery when compared to the untreated (control) hearts (78). These novel results suggested that the putative mK_{Ca} channels may be opened during early reperfusion and intrinsically contribute to reducing mitochondrial damage and concomitant cellular injury. Therefore, effective therapeutic targeting of these mitochondrial channels during reperfusion could represent additional valuable approach that could have clinical utility during I/R procedures. In this case, mK_{Ca} channel agonists could be used as adjuvant therapy with other mitochondrial targeted approaches to provide further protection against oxidative/nitrosative damage.

It is noteworthy that conventional pre- and post-conditioning provide minimal cardioprotection in the aged rat (1,76) and in human hearts (50). The lack of conventional cardioprotection in the aged heart could be attributed to the decline in mitochondrial function and possible imbalances in ROS generation and scavenging, with greater net ROS emission. Indeed, in the aged heart, ischemic damage to mitochondria is superimposed upon aging-induced defects in mitochondrial oxidative metabolism (43,44,46) as a consequence of decreases in complex I and IV activities (23) with concomitant increase in ROS emission. The aged heart generally sustains increased damage during I/R (8,26,42). Older patients with ischemic heart disease generally have impaired recovery of myocardial function after cardiac surgery or other cardiac interventions when compared to younger patients (61).

Insofar as the aged heart could not be pre- or post-conditioned, other mitochondria-targeted approaches have shown potential for cardioprotection. For example, nutrition fortified with acetylcarnitine has been shown to restore cardiolipin and mitochondrial function in aged hearts (12,45). It has been reported that blocking complex I before ischemia, unlike preconditioning, protects the aging heart from I/R injury and protects mitochondria (45). These observations provide strong empirical evidence for the association of aging-related defects in mitochondrial metabolism to the enhanced damage in old hearts. This also highlights the importance of considering other approaches to modulate mitochondrial oxidative metabolism to protect the aged heart (15).

Conclusion

Most of this review has concentrated on defined approaches, i.e. pre- and post-conditioning and some direct approaches, to alter mitochondrial function in treating or preventing I/R injury. Clearly, the efficacies of these approaches remain to be resolved. Furthermore, targeting mitochondria for specific therapeutic purposes could pose a dilemma of how to protect, while at the same time, preserve normal cellular function. Different cells and tissues have distinct sensitivities and responses to mitochondrial dysfunction (15). Appreciation of these differences will be important when considering mitochondrial therapeutic strategies in a more integrated setting than in an isolated cells, isolated mitochondria or in an isolated heart model. Nonetheless, most current efforts are focused on preventing mitochondrial oxidative damage that arises from deleterious conditions. These include decreasing mPTP opening, decreasing DY_m to reduce $O_2^{\bullet-}$ production, and limiting mCa^{2+} overload. Pre- and post-conditioning have both minimized mitochondrial damage and attenuated cell injury by targeting the above mitochondrial variables, directly or indirectly. However, proper design and refinement of current mitochondriotropic drugs may seem to be the most promising approach to prevent or treat mitochondrial dysfunction like cardiac I/R injury, as we have stressed in this review. As noted previously (15), much work is required to develop and improve mitochondria-directed strategies that would lead to better protection of the heart against I/R injury. Any such targeted approach should have the potential for clinical translation.

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References

1. Abete P, Testa G, Ferrara N, De Santis D, Capaccio P, Viati L, Calabrese C, Cacciatore F, Longobardi G, Condorelli M, Napoli C, Rengo F. Cardioprotective effect of ischemic preconditioning is preserved in food-restricted senescent rats. Am J Physiol Heart Circ Physiol 282: H1978-87, 2002.

2. Aldakkak M, Camara A, Heisner J, Gadicherla A, Boelens A, Dash R, Stowe D. Ranolazine delays Ca^{2+}-induced mitochondrial permeability transition pore opening and membrane potential depolarization in guinea pig heart mitochondria. FASEB J Abstract, 2010.

3. 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: 406-15, 2008.

4. Aldakkak M, Stowe DF, Cheng Q, Kwok WM, Camara AK. Mitochondrial matrix K^+ flux independent of large-conductance Ca^{2+}-activated K^+ channel opening. Am J Physiol Cell Physiol 298: C530-41, 2010.

5. Aldakkak M, Stowe DF, Heisner JS, Spence M, Camara AK. Enhanced Na^+/H^+ exchange during ischemia and reperfusion impairs mitochondrial bioenergetics and myocardial function. J Cardiovasc Pharmacol 52: 236-44,

2008.
6. Aldakkak M, Stowe DF, Lesnefsky EJ, Heisner JS, Chen Q, Camara AK. Modulation of mitochondrial bioenergetics in the isolated Guinea pig beating heart by potassium and lidocaine cardioplegia: implications for cardioprotection. Journal of Cardiovascular Pharmacology 54: 298-309, 2009.
7. Andreyev AY, Kushnareva YE, Starkov AA. Mitochondrial metabolism of reactive oxygen species. Biochemistry (Mosc) 70: 200-14 [review], 2005.
8. Azhar G, Gao W, Liu L, Wei JY. Ischemia-reperfusion in the adult mouse heart influence of age. Exp Gerontol 34: 699-714, 1999.
9. Brooks C, Wei Q, Cho SG, Dong Z. Regulation of mitochondrial dynamics in acute kidney injury in cell culture and rodent models. Journal of Clinical Investigation 119: 1275-85, 2009.
10. Burelle Y, Khairallah M, Ascah A, Allen BG, Deschepper CF, Petrof BJ, Des Rosiers C. Alterations in mitochondrial function as a harbinger of cardiomyopathy: lessons from the dystrophic heart. Journal of Molecular and Cellular Cardiology 48: 310-21, 2009.
11. Cain BS, Meldrum DR, Joo KS, Wang JF, Meng X, Cleveland JC, Jr., Banerjee A, Harken AH. Human SERCA2a levels correlate inversely with age in senescent human myocardium. Journal of the American College of Cardiology 32: 458-67, 1998.
12. Calabrese V, Ravagna A, Colombrita C, Scapagnini G, Guagliano E, Calvani M, Butterfield DA, Giuffrida Stella AM. Acetylcarnitine induces heme oxygenase in rat astrocytes and protects against oxidative stress: involvement of the transcription factor Nrf2. J Neurosci Res 79: 509-21, 2005.
13. Camara A, 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: C2021-31, 2007.
14. Camara AK, Begic Z, Kwok WM, Bosnjak ZJ. Differential modulation of the cardiac L- and T-type calcium channel currents by isoflurane. Anesthesiology 95: 515-24, 2001.
15. Camara AK, Lesnefsky EJ, Stowe DF. Potential Therapeutic Benefits of Strategies Directed to Mitochondria. Antioxid Redox Signal 13: 279-347, 2010.
16. Camello-Almaraz C, Gomez-Pinilla PJ, Pozo MJ, Camello PJ. Mitochondrial reactive oxygen species and Ca^{2+} signaling. Am J Physiol Cell Physiol 291: C1082-8 [review], 2006.
17. Chan D, Frank S, Rojo M. Mitochondrial dynamics in cell life and death. Cell Death and Differentiation 13: 680-4, 2006.
18. Chan DC. Mitochondria: dynamic organelles in disease, aging, and development. Cell 125: 1241-52, 2006.
19. Chazotte B, Vanderkooi G. Multiple sites of inhibition of mitochondrial electron transport by local anesthetics. Biochim Biophys Acta 636: 153-61, 1981.
20. Chen Q, Camara AK, Stowe DF, Hoppel CL, Lesnefsky EJ. Modulation of electron transport protects cardiac mitochondria and decreases myocardial injury during ischemia and reperfusion [Review]. Am J Physiol Cell Physiol 292: C137-47 [review], 2007.
21. Cheng Q, Bienengraeber M, Bosnjak Z, Kwok W. Purification of Human Mitochondrial Adenine Nucleotide Translocator: Implications in Preconditioning. Anesthesiology, 2008.
22. Chipuk JE, Bouchier-Hayes L, Green DR. Mitochondrial outer membrane permeabilization during apoptosis: the innocent bystander scenario. Cell Death Differ 13: 1396-402, 2006.
23. Cooper JM, Mann VM, Schapira AH. Analyses of mitochondrial respiratory chain function and mitochondrial DNA deletion in human skeletal muscle: effect of ageing. J Neurol Sci 113: 91-8, 1992.
24. Erusalimsky JD, Moncada S. Nitric oxide and mitochondrial signaling: from physiology to pathophysiology. Arterioscler Thromb Vasc Biol 27: 2524-31, 2007.
25. Feng J, Zhu M, Schaub MC, Gehrig P, Roschitzki B, Lucchinetti E, Zaugg M. Phosphoproteome analysis of isoflurane-protected heart mitochondria: phosphorylation of adenine nucleotide translocator-1 on Tyr194 regulates mitochondrial function. Cardiovasc Res 80: 20-9, 2008.
26. Frolkis VV, Frolkis RA, Mkhitarian LS, Fraifeld VE. Age-dependent effects of ischemia and reperfusion on cardiac function and Ca2+ transport in myocardium. Gerontology 37: 233-9, 1991.
27. Garcia-Dorado D, Rodriguez-Sinovas A, Ruiz-Meana M, Inserte J, Agullo L, Cabestrero A. The end-effectors of preconditioning protection against myocardial cell death secondary to ischemia-reperfusion. Cardiovascular Research 70: 274-85, 2006.
28. Garlid KD, Jaburek M, Jezek P, Varecha M. How do uncoupling proteins uncouple? [short review]. Biochim Biophys Acta 1459: 383-9, 2000.
29. Ge ZD, Pravdic D, Bienengraeber M, Pratt PF, Jr., Auchampach JA, Gross GJ, Kersten JR, Warltier DC. Isoflurane postconditioning protects against reperfusion injury by preventing mitochondrial permeability transition by an endothelial nitric oxide synthase-dependent mechanism. Anesthesiology 112: 73-85.
30. Girotti AW. Lipid hydroperoxide generation, turnover, and effector action in biological systems. Journal of Lipid Research 39: 1529-42, 1998.
31. Gottlob K, Majewski N, Kennedy S, Kandel E, Robey RB, Hay N. Inhibition of early apoptotic events by Akt/PKB is dependent on the first committed step of glycolysis and mitochondrial hexokinase. Genes Dev 15: 1406-18, 2001.
32. Gross GJ, Peart JN. Opioids and myocardial reperfusion injury. Arch Mal Coeur Vaiss 100: 231-7, 2007.
33. Halestrap AP, Clarke SJ, Javadov SA. Mitochondrial permeability transition pore opening during myocardial reperfusion--a target for cardioprotection. Cardiovasc Res 61: 372-85, 2004.
34. Hanley PJ, Daut J. K_{ATP} channels and preconditioning: a re-examination of the role of mitochondrial K_{ATP} channels and an overview of alternative mechanisms. J Mol Cell Cardiol 39: 17-50, 2005.
35. Jou M, Peng T. Visualization of melatonin's multiple mitochondrial levels of protection against mitochondrial Ca^{2+} mediated permeability transition and beyond in rat brain astrocytes. Biophysical Journal [Abstract]: 1953-Pos, 2010.
36. Kawata H, Yoshida K, Kawamoto A, Kurioka H, Takase E, Sasaki Y, Hatanaka K, Kobayashi M, Ueyama T, Hashimoto T, Dohi K. Ischemic preconditioning upregulates vascular endothelial growth factor mRNA expression and neovascularization via nuclear translocation of protein kinase C epsilon in the rat ischemic myocardium. Circ Res 88: 696-704, 2001.
37. 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: H566-74, 2003.
38. Kevin LG, Novalija E, Riess ML, Camara AK, Rhodes SS, Stowe DF. Sevoflurane exposure generates superoxide but leads to decreased superoxide during ischemia and reperfusion in isolated hearts. Anesth Analg 96: 949-55, 2003.
39. Kin H, Zatta AJ, Lofye MT, Amerson BS, Halkos ME, Kerendi F, Zhao ZQ, Guyton RA, Headrick JP, Vinten-Johansen J. Postconditioning reduces infarct size via adenosine receptor activation by endogenous adenosine. Cardiovasc Res 67: 124-33, 2005.
40. Lebuffe G, Schumacker PT, Shao ZH, Anderson T, Iwase H, Vanden Hoek TL. ROS and NO trigger early preconditioning: relationship to mitochondrial K_{ATP} channel. Am J Physiol Heart Circ Physiol 284: H299-308, 2003.
41. Lenaz G, Genova ML. Structure and organization of mitochondrial respiratory complexes: a new understanding of an old subject. Antioxid Redox Signal 12: 961-1008, 2010.
42. Lesnefsky EJ, Gallo DS, Ye J, Whittingham TS, Lust WD. Aging increases ischemia-reperfusion injury in the isolated, buffer-perfused heart. J Lab Clin Med 124: 843-51, 1994.
43. Lesnefsky EJ, Gudz TI, Migita CT, Ikeda-Saito M, Hassan MO, Turkaly PJ, Hoppel CL. Ischemic injury to mitochondrial electron transport in the aging heart: damage to the iron-sulfur protein subunit of electron transport complex III. Arch Biochem Biophys 385: 117-28, 2001.
44. Lesnefsky EJ, Gudz TI, Moghaddas S, Migita CT, Ikeda-Saito M, Turkaly PJ, Hoppel CL. Aging decreases electron transport complex III activity in heart interfibrillar mitochondria by alteration of the cytochrome c binding site. J Mol Cell Cardiol 33: 37-47, 2001.
45. Lesnefsky EJ, He D, Moghaddas S, Hoppel CL. Reversal of mitochondrial defects before ischemia protects the aged heart. Faseb J 20: 1543-5, 2006.

46. Lesnefsky EJ, Moghaddas S, Tandler B, Kerner J, Hoppel CL. Mitochondrial dysfunction in cardiac disease: ischemia--reperfusion, aging, and heart failure. J Mol Cell Cardiol 33: 1065-89, 2001.
47. Maddock HL, Mocanu MM, Yellon DM. Adenosine A(3) receptor activation protects the myocardium from reperfusion/reoxygenation injury. Am J Physiol Heart Circ Physiol 283: H1307-13, 2002.
48. Meister A. Glutathione biosynthesis and its inhibition. Methods Enzymol 252: 26-30, 1995.
49. Meister A. Glutathione metabolism. Methods Enzymol 251: 3-7, 1995.
50. Mio Y, Bienengraeber MW, Marinovic J, Gutterman DD, Rakic M, Bosnjak ZJ, Stadnicka A. Age-related attenuation of isoflurane preconditioning in human atrial cardiomyocytes: roles for mitochondrial respiration and sarcolemmal adenosine triphosphate-sensitive potassium channel activity. Anesthesiology 108: 612-20, 2008.
51. Mockford KA, Girn HR, Homer-Vanniasinkam S. Postconditioning: Current Controversies and Clinical Implications. Eur J Vasc Endovasc Surg, 2009.
52. Novalija E, Varadarajan SG, Camara AKS, An JZ, Chen Q, Riess ML, Hogg H, Stowe DF. Anesthetic preconditioning: Triggering role of reactive oxygen and nitrogen species in isolated hearts. Am J Physiol Heart Circ Physiol 283: H44-52, 2002.
53.O'Rourke B.Mitochondrial ion channels.AnnuRevPhysiol69:19-49, 2007.
54. O'Rourke B, Cortassa S, Aon MA. Mitochondrial ion channels: gatekeepers of life and death. Physiology (Bethesda) 20: 303-15, 2005.
55. Pagel PS. Postconditioning by volatile anesthetics: salvaging ischemic myocardium at reperfusion by activation of prosurvival signaling. J Cardiothorac Vasc Anesth 22: 753-65, 2008.
56. Pagel PS,Krolikowski JG,Amour J,Warltier DC,Weihrauch D.Morphine Reduces the Threshold of Helium Preconditioning Against Myocardial Infarction: The Role of Opioid Receptors in Rabbits. J Cardiothorac Vasc Anesth.
57. Patel HH, Gross GJ. Diazoxide induced cardioprotection: what comes first, K(ATP) channels or reactive oxygen species? Cardiovascular Research 51: 633-6, 2001.
58. Peixoto PM, Ryu SY, Kinnally KW. Mitochondrial ion channels as therapeutic targets. FEBS Letters 584: 2142-52, 2010.
59. Pfanner N, Wiedemann N, Meisinger C, Lithgow T. Assembling the mitochondrial outer membrane. Nat Struct Mol Biol 11: 1044-8, 2004.
60. Pravdic DB, M Sedlic, F, Stadnicka A, Bosnjak, Z. Intracellular Ca2+ and pH modulation of in cardiomyocytes during postconditioning. Anesthesiology 109: A-859, 2008.
61. Reynen K, Bachmann K. Coronary arteriography in elderly patients: risk, therapeutic consequences and long-term follow-up. Coron Artery Dis 8: 657-66, 1997.
62. Rousset S, Alves-Guerra MC, Mozo J, Miroux B, Cassard-Doulcier AM, Bouillaud F, Ricquier D. The biology of mitochondrial uncoupling proteins. Diabetes 53 Suppl 1: S130-5, 2004.
63. Santos CH, Pontes JC, Miiji LN, Nakamura DI, Galhardo CA, Aguena SM. Postconditioning effect in the hepatic ischemia and reperfusion in rats. Acta Cir Bras 25: 163-8.
64. Schimke I, Schikora M, Meyer R, Dubel HP, Modersohn D, Kleber FX, Baumann G. Oxidative stress in the human heart is associated with changes in the antioxidative defense as shown after heart transplantation. Mol Cell Biochem 204: 89-96, 2000.
65. Schulz R, Boengler K, Totzeck A, Luo Y, Garcia-Dorado D, Heusch G. Connexin 43 in ischemic pre-and postconditioning.Heart Fail Rev 12:261-6, 2007.
66. Schwertz H, Carter JM, Abdudureheman M, Russ M, Buerke U, Schlitt A, Muller-WerdanU,Prondzinsky R, Werdan K, Buerke M. Myocardial ischemia/reperfusion causes VDAC phosphorylation which is reduced by cardioprotection with a p38 MAP kinase inhibitor. Proteomics 7: 4579-88, 2007.
67. Sedlic F, Sepac A, Pravdic D, Camara AK, Bienengraeber M, Brzezinska AK, Wakatsuki T, Bosnjak ZJ. Mitochondrial Depolarization Underlies Delay in Permeability Transition by Preconditioning with Isoflurane: Roles of ROS and Ca2+. Am J Physiol Cell Physiol, 2010.
68. Sharif MA, Bayraktutan U, Arya N, Badger SA, O'Donnell ME, Young IS, Soong CV. Effects of antioxidants on endothelial function in human saphenous vein in an ex vivo model. Angiology 60: 448-54, 2009.
69. Solaini G, Harris DA. Biochemical dysfunction in heart mitochondria exposed to ischaemia and reperfusion. Biochem J 390: 377-94, 2005.
70. Stowe DF, Aldakkak M, Camara AKS, Riess ML, Heinen A, Varadarajan SG, Jiang MT. Cardiac mitochondrial preconditioning by big Ca^{2+}-sensitive K^+ channel opening requires superoxide radical generation. Am J Physiol Heart Circ Physiol 290: H434-H440, 2006.
71. Stowe DF, Camara AKS. Mitochondrial reactive oxygen species production in excitable cells: modulators of mitochondrial and cell function Antioxidants & Redox Signaling 11: 1373-1396 [Comprehensive Review], 2009.
72. Stowe DF, Riess ML. Reactive oxygen species and cardiac preconditioning: many questions remain. Cardiovasc Drugs Ther 18: 87-90 [short review], 2004.
73. Szeto HH. Mitochondria-targeted peptide antioxidants: novel neuroprotective agents. Aaps J 8: E521-31, 2006.
74.SzetoHH.Development of mitochondria-targeted aromatic-cationic peptidesfor neurodegenerative diseases.AnnNYAcadSci1147:112-21, 2008.
75. Szewczyk A, Wojtczak L. Mitochondria as a pharmacological target. Pharmacol Rev 54: 101-27, 2002.
76. Tani M, Honma Y, Hasegawa H, Tamaki K. Direct activation of mitochondrial K(ATP) channels mimics preconditioning but protein kinase C activation is less effective in middle-aged rat hearts. Cardiovasc Res 49: 56-68.
77. Valen G, Vaage J. Pre- and postconditioning during cardiac surgery. Basic Res Cardiol 100: 179-86, 2005.
78. Varadarajan SC, AKS Rhodes, SS Aldakkak, M Heisner, JS and Stowe DF. Cardiac mitochondrial Ca^{2+} -dependent big K^+ channels are open during reperfusion. FASEB J 21: A1224, 2007.
79. Vinten-Johansen J, Yellon DM, Opie LH. Postconditioning: a simple, clinically applicable procedure to improve revascularization in acute myocardial infarction. Circulation 112: 2085-8, 2005.
80. Wenner CE. Cell signaling and cancer-possible targets for therapy. Journal of Cellular Physiology 223: 299-308.
81. Yang CC, Lin LC, Wu MS, Chien CT, Lai MK. Repetitive hypoxic preconditioning attenuates renal ischemia/reperfusion induced oxidative injury via upregulating HIF-1 alpha-dependent bcl-2 signaling. Transplantation 88: 1251-60, 2009.
82. Yang XM, Proctor JB, Cui L, Krieg T, Downey JM, Cohen MV. Multiple, brief coronary occlusions during early reperfusion protect rabbit hearts by targeting cell signaling pathways. J Am Coll Cardiol 44: 1103-10, 2004.
83. Yellon DM, Downey JM. Preconditioning the myocardium: from cellular physiology to clinical cardiology. Physiological Reviews 83: 1113-51, 2003.
84. Zhao Y, Wang ZB, Xu JX. Effect of cytochrome c on the generation and elimination of $O_2^{\cdot-}$ and H_2O_2 in mitochondria.J Biol Chem 278: 2356-60, 2003.
85. Zuurbier CJ, Smeele KM, Eerbeek O. Mitochondrial hexokinase and cardioprotection of the intact heart. Journal of Bioenergetics and Biomembranes 41: 181-5, 2009.