[Frontiers in Bioscience 11, 2554-2564, September 1, 2006]

Mitochondrial Ca^{2+} transport, permeability transition and oxidative stress in cell death: implications in cardiotoxicity, neurodegeneration and dyslipidemias

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1. ABSTRACT

Mitochondrial Ca^{2+} transport is important in the maintenance of intracellular ion homeostasis, and also a key factor in the pathogenesis of many diseases. We discuss here the main aspects of mitochondrial Ca^{2+} transport, and how this transport is linked to changes in energy metabolism and redox state. Mitochondrial permeability transition, a consequence of excessive mitochondrial Ca^{2+} accumulation associated with oxidative stress is also discussed. Finally, our current understanding of the involvement of these mitochondrial processes in cardiac ischemia-reperfusion, neurodegeneration and dyslipidemias is presented.

2. INTRODUCTION

A number of new proteins and modulator metabolites have been recently detected in mitochondria, steering these organelles into the spotlight in studies concerning cell signaling, cell injury, and cell death. Understanding the molecular mechanisms of ATP synthesis, electron transfer reactions and biological roles of these newly discovered mitochondrial components has provided new insights into mitochondrial physiology. Evidence has been provided that mitochondria compromise one of the main pathways for apoptosis in vertebrate cells (see refs. 1-6, for recent reviews) and that dysfunctional mitochondria with decreased electron transfer rates and increased production rates of O_2^- and H_2O_2, both chemical species called reactive oxygen species (ROS), are associated with neurodegenerative diseases such as Huntington´s, Parkinson´s and Alzheimer´s diseases (7,8).

3. MITOCHONDRIAL FUNCTION AND Ca^{2+} TRANSPORT

Mitochondria are known to be the site of important metabolic pathways such as the citric acid cycle, fatty acid beta oxidation and amino acid oxidation. These organelles are also the site of oxidative phosphorylation, which is dependent on coupling between respiration, the transmembrane proton electrochemical potential and ADP phosphorylation, as postulated by Peter Mitchell (9). This transmembrane potential is generated by pumping protons across the inner mitochondrial membrane, while electrons flow through respiratory chain complexes. Proton pumping makes the matrix slightly alkaline and strongly negatively charged relative to the intermembrane space, and provides energy for ATP synthesis. Increases in the inner membrane

permeability to protons disrupt the proton electrochemical potential and may be a key event in many mitochondrially-mediated diseases. The continuous reduction of O_2 by the mitochondrial electron transport chain to build up the transmembrane electrical proton potential ($\Delta\Psi$) has a side effect: ROS generation (10-14).

As a result of the inner membrane electrochemical gradient, mitochondria are capable of accumulating large quantities of cations, as long as transporters or channels are present to transport the ions into the matrix. For example, mitochondria take up Ca^{2+} ions using $\Delta\Psi$ as driving force and through the activity of inner membrane Ca^{2+} channels, accumulating large quantities of the cation (15). This effect was described more than 40 years ago (16, 17), but for a long time mitochondria were not believed to accumulate Ca^{2+} under physiological conditions, since the affinity of the channel is significantly lower than the average intracellular Ca^{2+} concentrations (15). However, more recent studies using mitochondrially-targeted aequorin (18,19) showed that mitochondria do, in fact, have an important role in physiological intracellular Ca^{2+} homeostasis, and take up Ca^{2+} due to the existence of microenvironments with high concentrations of this ion located near these organelles. This uptake is necessary to control the activities of key intramitochondrial enzymes, including pyruvate, isocitrate and α-ketoglutarate dehydrogenases (20). In addition to this role in intracellular signaling, mitochondria also act as high capacity intracellular Ca^{2+} stores under pathological conditions (15). High uptake of Ca^{2+} by mitochondria can lead to the non-selective inner membrane permeabilization know as mitochondrial permeability transition (MPT).

Ca^{2+} uptake into mitochondria occurs mostly due to ion fluxes through a selective ruthenium red-sensitive inner membrane Ca^{2+} channel (15,21). In addition, Ca^{2+} uptake through a mitochondrial ryanodine receptor has been reported (22). Initially, Ca^{2+} uptake by mitochondria was thought to occur exclusively in vertebrates. Latter it was observed that plants, trypanosomes and other primitive eukaryotes share this same property with some specific features (23). Based on kinetic studies, Gunter´s group identified two modes of Ca^{2+} uptake: standard and rapid (24), but it is not known if these forms of uptake are promoted by distinct molecular components or different activities of the same channel. Ca^{2+} efflux from the mitochondrial matrix occurs through Ca^{2+}/Na^{+} and Ca^{2+}/H^{+} exchangers. Ca^{2+}/Na^{+} exchange is dominant in heart, brain, skeletal muscle, and brown fat, while Ca^{2+}/H^{+} exchange is dominant in liver, kidney, lung and smooth muscle (25).

Evidence has been provided that alterations in mitochondrial Ca^{2+} homeostasis are linked to cellular oxidative stress and may be causes of cell death under a variety of pathological conditions. We will discuss here the mechanisms in which Ca^{2+} homeostasis and oxidative stress are linked, and present a few pathological conditions in which mitochondrial ion transport and redox state determine cell survival and death.

4. MITOCHONDRIAL REACTIVE OXYGEN SPECIES GENERATION AND OXIDATIVE STRESS

The generation of ROS by mitochondria is a continuous and physiological event. Depending on the tissue, respiratory conditions and the substrates used, 0.02 - 2% of the total mitochondrial O_2 consumption results in the generation of the superoxide anion (O_2^{-}) *in vitro* (10,26,27). The process is due to the monoelectronic reduction of O_2 at NADH:coenzyme Q, succinate:coenzyme Q and coenzyme QH_2:cytochrome c oxido-reductases (complexes I, II and III) of the respiratory chain (10-14). These respiratory complexes are located in the inner mitochondrial membrane and O_2^{-} production occurs at the matrix side in respiratory complexes I and II and at the matrix and intermembrane space in respiratory complex III (11,12). There is also recent evidence that O_2^{-} generation is promoted by matrix-soluble dehydrogenases such as pyruvate and α-ketoglutarate dehydrogenases (27,28).

The free radical O_2^{-} presents moderate chemical reactivity in aqueous solutions, and can suffer many different reactions within mitochondria, generating other products of the partial reduction of O_2, such as H_2O_2 and hydroxyl radical ($HO^{\cdot}$). Superoxide dismutases, present both in the mitochondrial matrix (Mn-SOD) (29,30) and in the intermembrane space (Cu,Zn-SOD) (31,32), catalyze the generation of H_2O_2 from O_2^{-} dismutation. H_2O_2 is a more stable and membrane-permeable chemical species, which can be removed by different enzymes with peroxidase activity. Alternatively, H_2O_2 can generate $HO^{\cdot}$, the highly oxidative and cytotoxic ROS, through reductive homolytic cleavage. Most of the $HO^{\cdot}$ generated in vivo probably comes from the iron-dependent breakdown of H_2O_2, via Fenton´s reaction ($H_2O_2 + Fe^{2+} => HO^{-} + HO^{\cdot} + Fe^{3+}$) (33).

Glutathione peroxidase (GPx) (34) was the first thiol antioxidant enzyme characterized in mitochondria (35). GPx removes H_2O_2 at the expense of reduced glutathione (GSH), generating oxidized glutathione (GSSG). GSSG is then reduced by glutathione reductase, using NADPH as an electron source. The generated $NADP^{+}$ is reduced by electrons derived from NADH by mitochondrial $NAD(P)^{+}$ transhydrogenase (36). Cytoplasmic redox state, pyruvate dehydrogenase and citric acid cycle activity, respiratory rates and high-energy phosphate levels determine mitochondrial NADH levels, providing an important link between energy metabolism and mitochondrial ROS removal.

Mitochondrial H_2O_2 removal is also conducted by catalase (described to date only in heart mitochondria; 37) and thioredoxin peroxidase (TPx), another thiol-dependent antioxidant (38). TPx acts similarly to GPx, but using reduced thioredoxin as a substrate. Thioredoxin reductase then recovers oxidized thioredoxin, using NADPH as an electron source. TPx is abundant in mitochondria chronically submitted to oxidative stress, such as those in the adrenal cortex (39) and is able to inhibit ROS-mediated apoptotic cell death (40). We have also showed that

exogenous TPx added to isolated mitochondria (41, 42) and endogenous cytosolic and mitochondrial *Saccharomyces cerevisiae* TPx (43) protect mitochondria against protein thiol oxidation and inner membrane permeabilization.

Mitochondria also generate the reactive nitrogen species (RNS) nitric oxide (NO), a relatively stable and membrane permeable free radical involved in many signaling pathways (see ref. 44 for review). The existence of a mitochondrial NO synthase (mtNOS) was first suggested by immunohistochemical studies that found that anti-NOS antibodies stained mitochondria (45-47). Later, the biochemical aspects of mtNOS were extensively characterized (48-50), although there is still considerable debate as to the molecular identity of mtNOS (51-53). Interestingly, mtNOS is activated by Ca^{2+} (48, 54), as observed with other NOS, providing another link between mitochondrial Ca^{2+} transport and redox state.

The main role of NO is believed to be the regulation of electron transfert, since NO reversibly inhibits cytochrome oxidase, the terminal oxidase of the electron transfer chain (51-53). As a result of this inhibition, ATP synthesis should decrease, local O_2 tensions rise and H_2O_2 release enhanced, effects that are not necessarily deleterious. For example, in fireflies, the resulting increase in local O_2 and H_2O_2 levels regulates light production (55). Another important role for NO is to regulate mitochondrial biogenesis in a manner dependent on cGMP (56). Furthermore, there is emerging evidence that the S-nitrosylation by NO of various mitochondrial proteins has an important role in intracellular signaling processes (57,58).

Because mitochondria generate both NO and $O_2^{\cdot-}$, it is expected that these two species would react generating peroxynitrite, as reported by several groups (59-63). Peroxynitrite is a highly reactive RNS, which promotes tyrosine nitration and S-nitrosation, reacting with electron transfer components and glutathione (63). In addition, peroxynitrite can react with CO_2, present abundantly in the intracellular environment, generating the highly reactive carbonate radical (64).

Mitochondrially-generated ROS and RNS can oxidize macromolecules both in mitochondria themselves and from other intracellular locations. Indeed, lipid peroxidation and DNA damage by mitochondrially-generated ROS has been extensively documented (11, 65-68). Proteins, in particular those of the inner membrane, are primary targets for the oxidative damage induced by mitochondrially-generated ROS and RNS, because the inner membrane is extremely rich in proteins, which compose more than 80% of its dry weight. Much of the oxidative damage in mitochondrial membrane proteins involves thiol oxidation and carbonyl formation. Carbonyl formation and thiol oxidation result in mitochondrial impairment with respiratory chain inhibition (69,70) and non-selective inner membrane permeabilization due to MPT (11,71,72).

5. MITOCHONDRIAL PERMEABILITY TRANSITION

MPT is a non-selective permeabilization of the inner mitochondrial membrane typically promoted by oxidative stress and the accumulation of excessive quantities of Ca^{2+} ions, in a process that is stimulated by a variety of compounds or conditions (25,71-74). Inner membrane permeabilization caused by MPT results in loss of matrix components, impairment of mitochondrial function and substantial mitochondrial swelling, with consequent outer membrane rupture and release of intermembrane space proteins (4,71-75). As a result, MPT actively participates in events that initiate either necrotic or apoptotic cell death.

Despite extensive research, the nature of the membrane alterations that lead to MPT still remains a debated matter. MPT clearly involves membrane proteins, almost certainly a group of modified, missfolded and assembled inner membrane components (72,76). Possible components of the MPT pore include the adenine nucleotide translocator, VDAC, cyclophilin D, hexokinase, creatine kinase and the benzodiazepine receptor (77-79).

MPT is prevented by thiol reductants such as dithiothreitol (80), while thiol oxidants promote MPT, indicating that the protein modifications that induce MPT involve thiol oxidation (80-82). Interestingly, thiol cross linkage seems to be important for these conformational changes, since dithiol reagents promote MPT and cross-linked inner membrane proteins can be observed after MPT (71,80).

We observed that a wide variety of antioxidants protect against MPT caused by distinct conditions, suggesting that this process is a result of mitochondrial oxidative stress leading to thiol oxidation (41, 80, 83-86). Further evidence that MPT was caused by ROS was provided by the discovery that this process could be promoted through the addition of exogenous sources of ROS (87,88) and RNS (89).

The link between Ca^{2+}, a necessary trigger for MPT, and enhanced ROS may be related to changes in lipid organization of the inner mitochondrial membrane promoted by interactions with this ion (90, 91). Grijalba et al. (91) reported that the binding of Ca^{2+} to the inner face of the inner mitochondrial membrane leads to the formation of cardiolipin patches. These changes in lipid organization lead to enhanced ROS release by the mitochondrial respiratory chain, promoting inner membrane thiol oxidation and MPT. In addition, Ca^{2+} ions may modulate directly the open-closed state of the MPT pore, as indicated by experiments showing that Ca^{2+} is necessary even after thiol oxidation already occurred (92-94).

6. MITOCHONDRIA AND CELL DEATH

Because of their central role in energy metabolism, mitochondrial function is essential to maintain cellular integrity. Indeed, many conditions which lead to

mitochondrial damage with functional impairment such as inner membrane lipid peroxidation or MPT cause necrotic cell death (11,73,74,95,96).

In addition to causing necrosis, mitochondrial membrane permeabilization can lead to apoptosis, since these organelles contain proteins involved in this process such as cytochrome c, the apoptosis inducing factor and pro-caspases (75,97-99). The mitochondrial, or intrinsic apoptotic pathway can act independently of the cell surface death receptors-mediated extrinsic apoptosis pathway to lead to cell death, or may act as an amplifying step within the extrinsic pathway (100).

Mitochondrially-mediated apoptosis is initiated when proteins participating in this process are released from the organelle into the cytosol. Since apoptogenic mitochondrial proteins are located in the intermembrane space, this release usually involves only outer membrane permeabilization, a situation which has the clear advantage of maintaining inner membrane integrity and, thus, oxidative phosphorylation (74). Outer mitochondrial membrane permeabilization is a regulated process mediated by pro-apoptotic Bcl-2 family proteins including Bax, Bid and Bad (101-103).

In addition, mitochondria can also release apoptogenic factors after their inner membranes are damaged or permeabilized by MPT, leading to matrix swelling and outer membrane rupture (73-75,95). This form of apoptosis occurs when cells are damaged or stressed in some manner that induces MPT, and thus is often seen in association with necrotic cell death. Under these conditions, the determinant factor leading toward necrosis or apoptosis will be the ability to retain physiological intracellular ATP levels (6,74,104).

7. MITOCHONDRIA IN TISSUE DAMAGE FOLLOWING CARDIAC ISCHEMIA-REPERFUSION

Tissue damage caused by heart attack involves a lack of adequate O_2 and nutrient availability for the cardiac tissue (ischemia), followed by a return of O_2 and nutrients (reperfusion). The changes in energy metabolism and redox state that occur during ischemia-reperfusion include a set of conditions that typically lead to MPT: decreased ATP levels, increases in cytosolic phosphate and Ca^{2+} levels and augmented ROS release, which are secondary to abrupt increases in O_2 tensions (15,73). Indeed, MPT has been clearly demonstrated to participate in tissue damage occurring during cardiac reperfusion following ischemia by studies using radioactive deoxyglucose to measure mitochondrial swelling within intact hearts. Furthermore, tissue damage under these conditions is prevented by MPT inhibitor cyclosporin A (105). In addition, cardiac damage promoted by ischemia-reperfusion can be significantly decreased by the presence of antioxidants during reperfusion, confirming that oxidative stress is a cause of tissue damage under these conditions (106).

Cardiac damage promoted by ischemia-reperfusion can also be prevented by ischemic preconditioning, or a series of short ischemic periods preceding ischemia (107). In addition to ischemic preconditioning, cardiac tissues can also be preconditioned by mild treatments with normally damaging compounds such as H_2O_2 and Ca^{2+} (108,109). These studies, in addition to experiments using selective inhibitors of signaling processes, indicate that the mechanisms through which ischemic preconditioning leads to cardioprotection are complex, and involve signaling by kinases, ROS, RNS and changes in mitochondrial Ca^{2+} and K^+ homeostasis (110-112).

Several studies have demonstrated that preconditioning prevents MPT pore opening during reperfusion (109,113-115). The prevention of MPT by preconditioning is linked to the activation of ATP-sensitive inner mitochondrial membrane K^+ channels (mitoK_{ATP}; 112,114,116). These channels allow K^+ ions to flow into the mitochondrial matrix, at rates much slower than Ca^{2+} uptake rates. The result of increased K^+ uptake is a controlled increment in matrix volumes, which regulates the transport of ATP and ADP across mitochondrial membranes preserving the energy status of the tissue (112,117). In addition, mitoK_{ATP} activity prevents excessive mitochondrial Ca^{2+} loading that occurs during ischemia-reperfusion (113,118), resulting Ca^{2+}-induced ROS release (116). Indeed, we have found that the mild mitochondrial uncoupling promoted by mitoK_{ATP} activity is sufficient to decrease ROS release even in the absence of Ca^{2+} accumulation (119). Although this concept is at odds with data from other groups suggesting that mitoK_{ATP} activity increases ROS release (120,121), we have recently found that these studies were based on artifactual increases in dichlorofluorescein (a ROS indicator) fluorescence caused by the mitoK_{ATP} agonist diazoxide (122). The concomitant effects of mitoK_{ATP} activity, decreasing mitochondrial ROS release and preventing excessive Ca^{2+} accumulation under ischemic conditions, certainly explain the prevention of MPT when mitoK_{ATP} is activated by preconditioning or treatment with selective mitoK_{ATP} agonists (122).

8. MITOCHONDRIA AND NEURONAL DAMAGE

Since neurons are highly dependent on oxidative energy metabolism, they are uniquely sensitive to changes in oxidative phosphorylation. In fact, mitochondrial dysfunction has been implicated in neural cell death associated with various disorders including stroke, and Parkinson's and Huntington's diseases (123-126). Energy metabolism defects in the central nervous system cause increases in neuronal intracellular Ca^{2+} levels, either by directly impairing Ca^{2+} removal systems or by excessive glutamate receptor activation, in a process known as excitotoxicity (127). Excitotoxicity is a central nervous system process in which an increased glutamate release in response to hypoglycemia, ischemia, or trauma, for example, results in neuronal necrosis or apoptosis (128). This glutamate-mediated neuronal cell death is promoted mainly by activation of *N*-methyl-D-aspartate (NMDA)

receptors, with Ca^{2+} and Na^{+} influx through respective channels (127,129). While Na^{+} influx into neurons is associated with cellular swelling, Ca^{2+} influx is correlated with cellular toxicity (129). Under excitotoxic conditions, mitochondria are the main organelle responsible for Ca^{2+} sequestration, an event associated with neuronal cell death, as long as inner membrane potentials are maintained by respiration or ATP hydrolysis (130). However, the mechanism through which mitochondrial Ca^{2+} overload signals to neuronal cell death is still unclear. As discussed previously in this review, increased Ca^{2+} concentrations in the mitochondrial matrix may induce MPT. In fact, neuronal death following hypoglycemia and brain ischemia is prevented by MPT inhibitors (131,132). However, the participation of MPT in excitotoxicity is controversial (133-136). One possible explanation for the lack of or limited participation of MPT in several experimental models of excitotoxicity is the presence of high levels of endogenous inhibitors of this phenomenon, such as adenine nucleotides and Mg^{2+} (137). Probably, experimental conditions with more severe energy deprivation (131,132,138), where phosphocreatine, ATP and ADP are depleted, favor the participation of MPT in neuronal cell death.

In stroke, changes in mitochondrial functions may affect tissue survival even after O_2 and glucose return during reperfusion (139). Mitochondrial alterations reported prior to neuronal death after ischemia-reperfusion *in vivo* include impairment of respiratory chain complexes, inhibition of the pyruvate dehydrogenase complex, altered Ca^{2+} homeostasis, increased lipid peroxidation, MPT and the release of proapoptotic mitochondrial proteins (128). We observed that mitochondrially-generated ROS increased in isolated rat hippocampal mitochondria at 4 h and 48 h but not at 24 h of reperfusion after transient (10 min) global cerebral ischemia (140). Interestingly, brain damage after stroke and traumatic brain injury was diminished in mice overexpressing human uncoupling protein 2 (UCP-2) in the central nervous system (141). This neuroprotective effect is probably associated with mild mitochondrial uncoupling and decreased mitochondrial oxidative stress promoted by UCP-2. Moreover, expression of *Ucp2* was enhanced by a sublethal brain insult (141), indicating that UCP-2 may be important for ischemic preconditioning.

Parkinson's disease is characterized by rigidity, tremor and bradykinesia accompanied with loss of dopaminergic neurons in the substantia nigra. This disorder was linked to mitochondrial alterations, when 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) intoxication was discovered to cause this disease (142). MPTP causes complex I inhibition, also detected in idiopathic Parkinson's disease (125). Indeed, complex I inhibition by rotenone causes parkinsonism (143). The mechanism through which this inhibition causes dopaminergic neuron degeneration appears to involve changes in mitochondrial ROS release (143,144). We found that Ca^{2+} and complex I inhibition synergistically increase brain mitochondrial ROS release and neuronal death (145,146).

Recent work from our group has focused on neuronal dysfunction and death under situations of respiratory chain complex II (succinate dehydrogenase) inhibition. It is well characterized that succinate dehydrogenase inhibitors such as 3-nitropropionate and malonate can induce neurochemical, histological and clinical features of Huntington´s disease (123, 128). Methylmalonate (147), or its metabolites (148), also inhibit succinate dehydrogenase. Methylmalonate accumulates during methylmalonic acidemia, a disorder of branched amino acid and odd-chain fatty acids metabolism, involving a defect in the conversion of methylmalonyl-coenzyme A to succinyl-coenzyme A (149). It is proposed that neuronal damage by respiratory complex II inhibitors involves impairment of energy metabolism and oxidative stress (123,124,128). Indeed, we found that respiratory chain complex II inhibitors and Ca^{2+} promote MPT in isolated organelles as well as in cultured PC12 cells and freshly prepared brain slices (138). Interestingly, our recent study indicates that treatment with diazoxide, an agonist of mitoK_{ATP}, can prevent death promoted by treatment with methylmalonate in PC12 cells and freshly prepared rat brain slices (A.J. Kowaltowski, E.N. Maciel, M. Fornazari & R.F. Castilho, unpublished observations).

9. MITOCHONDRIAL INVOLVEMENT IN DYSLIPIDEMIAS

Stroke and heart attack are commonly caused by atherosclerotic alterations of vessels promoted by dyslipidemias such as primary or secondary hypercholesterolemia and hypertriglyceridemia. Indeed, atherosclerotic disease remains a leading cause of death in western societies (150).

Recent findings have shown that mitochondrial energy metabolism is altered in some conditions of secondary dyslipidemias. For example, alterations in circulating lipid levels by hormones (151), dietary fat (152), and intravenous heparin plus lipid infusion (153) cause changes in the expression of mitochondrial uncoupling proteins, which are involved in the regulation of energy metabolism (154,155) and in the rate of ROS release by mitochondria (141, 154-156). In addition, the expression of UCPs is also altered in other metabolic disorders in which dyslipidemia may be present, such as diabetes, obesity and the metabolic syndrome (157-159). However, all the above conditions present a very complex metabolic context, where it is very difficult to discriminate the key causative(s) factor(s).

By using genetically modified mice we showed, for the first time, alterations in mitochondrial bioenergetics and redox state in primary hyperlipidemia (160,161). These mice models are very useful to study the effects of elevated plasma lipid levels *per se,* without other metabolic confounding factors. Mice overexpressing the apolipoprotein CIII develop severe hypertriglyceridemia and high plasma levels of free fatty acids (162) but exhibit normal glucose homeostasis (163,164). We showed that liver mitochondria from these mice present higher resting respiration and susceptibility to MPT (160). Interesting, the

phosphorylating respiration rates and phosphorylation efficiency (ADP/O ratio) were preserved. Accordingly, these results were not related to the activity or expression of UCPs (160). We proposed that the faster resting respiration represents a regulated adaptation to oxidize excess of free fatty acids in transgenic mice liver cells.

It is well known that primary hypercholesterolemia is an independent and sufficient condition to cause atherosclerosis. Cumulative experimental evidences from the 80's up to now have reinforced the "oxidative modification hypothesis of atherogenesis" (165). It postulates that the disease is triggered by the low density lipoprotein (LDL) oxidation caused by ROS from circulating and vascular wall cells. However, it is not known where and how the oxidative stress condition is established. We found that hypercholesterolemic LDL receptor gene knockout mice have higher mitochondrial ROS production rates, associated with an increased susceptibility to MPT, in several tissues (161). In addition to increased ROS production, LDL receptor knockout cells (spleen lymphocytes) present about five-fold higher intracellular Ca^{2+} concentrations, which further contributes to higher susceptibility of MPT (G. Degasperi, B. Paim, H.C.F. Oliveira & A.E. Vercesi, unpublished observations). The increase in ROS release seems to be related to the necessity these animals have for *de novo* synthesis of triglycerides and cholesterol in each cell due to the lack of LDL uptake (161). Lipid synthesis substantially oxidizes NADPH, impairing the glutathione and thioredoxin peroxidase antioxidant systems (72). These findings provide the first evidence of how oxidative stress is generated in LDL receptor defective cells and suggest an explanation for increased LDL oxidation, cell death, and atherogenesis observed in familial hypercholesterolemia.

Treatment of hypercholesterolemia with statins (3-hydroxy-3-methylglutaryl-CoA reductase inhibitors) may also affect mitochondria, since isolated liver mitochondria from hypercholesterolemic LDL receptor knockout mice treated during 15 days with therapeutic doses of lovastatin presented a higher susceptibility to Ca^{2+}-induced MPT (166). In addition, *in vitro* experiments showed that lovastatin (10-80 μM) induces MPT in isolated liver and muscle mitochondria, with increased inner membrane protein thiol oxidation (166). This process was dose-dependent and was more potently triggered by hydrophobic statins. The ability of statins to induce MPT may explain statin-induced apoptosis observed in cultured cells (167, 168). These effects of statins on mitochondria might lead to cell injury or death contributing to the deleterious side effects, *e.g.* myotoxicity, rhabdomyolysis and liver toxicity reported in statin-treated patients (169).

10. PERSPECTIVES

As a result of the discovery that mitochondria participate not only in accidental but also in apoptotic cell death, interest in the investigation of the role of these organelles in cell survival has skyrocketed. Many new and exciting discoveries have been made in the last few years, and will certainly continue to appear in the near future. We believe that the understanding of the role of mitochondria in different metabolic syndromes (including dyslipidemias, obesity, insulin resistance and hypertension) will certainly be a focal point in these studies. Furthermore, a clearer understanding of the role of mitochondrial K^+ transport in cardiac tissue protection should be achieved. The exact nature of changes in ion transport, energy metabolism and redox state involved in different neurodegenerative disorders should be investigated. Finally, clear physiological and signaling roles for mitochondrial ROS and RNS should be a point of interest for new studies within the next years.

11. ACKNOWLEDGEMENTS

This work is supported by grants to A.E.V., A.J.K., H.C.F.O. and R.F.C. from the Brazilian agencies *Fundação de Amparo à Pesquisa no Estado de São Paulo* (FAPESP) and *Conselho Nacional de Pesquisa e Desenvolvimento* (CNPq) and to A.J.K. and R.F.C. from the U.S.A. National Institutes of Health (NIH).

12. REFERENCES

1. Crow MT, K. Mani, Y.J. Nam & R.N. Kitsis: The mitochondrial death pathway and cardiac myocyte apoptosis. *Circ Res* 95, 957-970 (2004)
2. Jiang X & X. Wang: Cytochrome c-mediated apoptosis. *Annu Rev Biochem* 73, 87-106 (2004)
3. Polster BM & G. Fiskum: Mitochondrial mechanisms of neural cell apoptosis. *J Neurochem* 90, 1281-1289 (2004)
4. Green DR & G. Kroemer: The pathophysiology of mitochondrial cell death. *Science* 305, 626-629 (2004)
5. Bouchier-Hayes L, L. Lartigue & D.D. Newmeyer: Mitochondria, pharmacological manipulation of cell death. *J Clin Invest* 115, 2640-2647 (2005)
6. Lemasters JJ: Dying a thousand deaths, redundant pathways from different organelles to apoptosis and necrosis. *Gastroenterology* 129, 351-360 (2005)
7. Browne SE, R.J. Ferrante & M.F. Beal: Oxidative stress in Huntington's disease. *Brain Pathol* 9, 147-163 (1999)
8. Beal MF: Mitochondrial dysfunction and oxidative damage in Alzheimer's and Parkinson's diseases and coenzyme Q10 as a potential treatment. *J Bioenerg Biomembr* 36, 381-386 (2004)
9. Mitchell P: Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature* 191, 144-148 (1961)
10. Boveris A: Mitochondrial production of superoxide radical and hydrogen peroxide. *Adv Exp Med Biol* 78, 67-82 (1977)
11. Kowaltowski AJ & A.E. Vercesi: Mitochondrial damage induced by conditions of oxidative stress. *Free Radic Biol Med* 26, 463-471 (1999)
12. Turrens JF: Mitochondrial formation of reactive oxygen species. *J Physiol* 552, 335-344 (2003)
13. Balaban RS, S. Nemoto & T. Finkel: Mitochondria, oxidants, and aging. *Cell* 120, 483-495 (2005)
14. Brookes PS: Mitochondrial H^+ leak and ROS generation, an odd couple. *Free Radic Biol Med* 38, 12-23 (2005)

15. Gunter TE, L. Buntinas, G.C. Sparagna & K.K. Gunter: The Ca^{2+} transport mechanisms of mitochondria and Ca^{2+} uptake from physiological-type Ca^{2+} transients. *Biochim Biophys Acta* 1366, 5-15 (1998)
16. Lehninger AL, C.S. Rossi & J.W. Greenwalt: Respiration-dependent accumulation of inorganic phosphate and Ca ions by rat liver mitochondria. *Biochem Biophys Res Commun* 10, 444-448 (1963)
17. Chance B: The energy-linked reaction of calcium with mitochondria. *J Biol Chem* 240, 2729-2748 (1965)
18. Rizzuto R, A.W. Simpson, M. Brini & T. Pozzan: Rapid changes of mitochondrial Ca2+ revealed by specifically targeted recombinant aequorin. *Nature* 358, 325-327 (1992)
19. Rizzuto R, M. Brini, M. Murgia & T. Pozzan: Microdomains with high Ca2+ close to IP3-sensitive channels that are sensed by neighboring mitochondria. *Science* 262, 744-747 (1993)
20. Hansford RG: Dehydrogenase activation by Ca2+ in cells and tissues. *J Bioenerg Biomembr* 23, 823-854 (1991)
21. Kirichok Y, G. Krapivinsky & D.E. Clapham: The mitochondrial calcium uniporter is a highly selective ion channel. *Nature* 427, 360-364 (2004)
22. Beutner G, V.K. Sharma, D.R. Giovannucci, D.I. Yule & S.S. Sheu: Identification of a ryanodine receptor in rat heart mitochondria. *J Biol Chem* 276, 21482-21488 (2001)
23. Vercesi AE: Ca2+ transport and oxidative damage of mitochondria. *Braz J Med Biol Res* 26, 441-457 (1993)
24. Buntinas L, K.K. Gunter, G.C. Sparagna & T.E. Gunter: The rapid mode of calcium uptake into heart mitochondria (RaM), comparison to RaM in liver mitochondria. *Biochim Biophys Acta* 1504, 248-261 (2001)
25. Gunter TE, D.I. Yule, K.K. Gunter, R.A. Eliseev & J.D. Salter: Calcium and mitochondria. *FEBS Lett* 567, 96-102 (2004)
26. St-Pierre J, J.A. Buckingham, S.J. Roebuck & M.D. Brand: Topology of superoxide production from different sites in the mitochondrial electron transport chain. *J Biol Chem* 277, 44784-44790 (2002)
27. Starkov AA, G. Fiskum, C. Chinopoulos, B.J. Lorenzo, S.E. Browne, M.S. Patel & M.F. Beal: Mitochondrial alpha-ketoglutarate dehydrogenase complex generates reactive oxygen species. *J Neurosci* 24, 7779-7788 (2004)
28. Tretter L & V. Adam-Vizi: Generation of reactive oxygen species in the reaction catalyzed by alpha-ketoglutarate dehydrogenase. *J Neurosci* 24, 7771-7778 (2004)
29. Weisiger RA & I. Fridovich: Superoxide dismutase. Organelle specificity. *J Biol Chem* 248, 3582-3592 (1973)
30. Fridovich I: Superoxide radical and superoxide dismutases. *Annu Rev Biochem* 64, 97-112 (1995)
31. Okado-Matsumoto A & I. Fridovich: Subcellular distribution of superoxide dismutases (SOD) in rat liver, Cu,Zn-SOD in mitochondria. *J Biol Chem* 276, 38388-38393 (2001)
32. Sturtz LA, K. Diekert, L.T. Jensen, R. Lill & V.C. Culotta: A fraction of yeast Cu,Zn-superoxide dismutase and its metallochaperone, CCS, localize to the intermembrane space of mitochondria. A physiological role for SOD1 in guarding against mitochondrial oxidative damage. *J Biol Chem* 276, 38084-38089 (2001)
33. Halliwell B & J.M. Gutteridge: Biologically relevant metal ion-dependent hydroxyl radical generation. An update. *FEBS Lett* 307, 108-112 (1992)
34. Flohe L, W.A. Gunzler & H.H. Schock: Glutathione peroxidase, a selenoenzyme. *FEBS Lett* 32, 132-134 (1973)
35. Green RC & P.J. O'Brien: The cellular localisation of glutathione peroxidase and its release from mitochondria during swelling. *Biochim Biophys Acta* 197, 31-39 (1970)
36. Kaufman . & N.O. Kaplan: Pyridine nucleotide transhydrogenase. VIII. Properties of the transhydrogenase reactions of an enzyme complex isolated from beef heart mitochondria. *J Biol Chem* 236 2133-2139 (1961)
37. Radi R, J.F. Turrens,. L.Y. Chang, K.M. Bush, J.D. Crapo & B.A. Freeman: Detection of catalase in rat heart mitochondria. *J Biol Chem* 266, 22028-22034 (1991)
38. Rhee SG, K.H. Kim, H.Z. Chae, M.B. Yim, K. Uchida, L.E. Netto & E.R. Stadtman: Antioxidant defense mechanisms, a new thiol-specific antioxidant enzyme. *Ann N Y Acad Sci* 738, 86-92 (1994)
39. Watabe S, H. Hasegawa, K. Takimoto, Y. Yamamoto & S.Y. Takahashi: Possible function of SP-22, a substrate of mitochondrial ATP-dependent protease, as a radical scavenger. *Biochem Biophys Res Commun* 213, 1010-1016 (1995)
40. Zhang P, B. Liu, S. W. Kang, M.S. Seo, S.G. Rhee & L.M. Obeid: Thioredoxin peroxidase is a novel inhibitor of apoptosis with a mechanism distinct from that of Bcl-2. *J Biol Chem* 272, 30615-30618 (1997)
41. Kowaltowski AJ, L.E. Netto & A.E. Vercesi: The thiol-specific antioxidant enzyme prevents mitochondrial permeability transition. Evidence for the participation of reactive oxygen species in this mechanism. *J Biol Chem* 273, 12766-12769 (1998)
42. Kowaltowski AJ, A.E. Vercesi, S.G. Rhee & L.E. Netto: Catalases and thioredoxin peroxidase protect Saccharomyces cerevisiae against Ca^{2+}-induced mitochondrial membrane permeabilization and cell death. *FEBS Lett* 473, 177-182 (2000)
43. Monteiro G, A.J. Kowaltowski, M.H. Barros & L.E. Netto: Glutathione and thioredoxin peroxidases mediate susceptibility of yeast mitochondria to Ca^{2+}-induced damage. *Arch Biochem Biophys* 425, 14-24 (2004)
44. Stamler JS: Redox signaling, nitrosylation and related target interactions of nitric oxide. *Cell* 78, 931-936 (1994)
45. Bates TE, A. Loesch, G. Burnstock & J.B. Clark: Immunocytochemical evidence for a mitochondrially located nitric oxide synthase in brain and liver. *Biochem Biophys Res Commun* 213, 896-900 (1995)
46. Kobzik L, B. Stringer, J.L. Balligand, M.B. Reid & J.S. Stamler: Endothelial type nitric oxide synthase in skeletal muscle fibers, mitochondrial relationships. *Biochem Biophys Res Commun* 211, 375-381 (1995)
47. Frandsen U, M. Lopez-Figueroa & Y. Hellsten: Localization of nitric oxide synthase in human skeletal muscle. *Biochem Biophys Res Commun* 227, 88-93 (1996)
48. Ghafourifar P & C. Richter: Nitric oxide synthase activity in mitochondria. *FEBS Lett* 418, 291-296 (1997)
49. Giulivi C, J.J. Poderoso & A. Boveris: Production of nitric oxide by mitochondria. *J Biol Chem* 273, 11038-11043 (1998)
50. Tatoyan A & C. Giulivi: Purification and characterization of a nitric-oxide synthase from rat liver mitochondria. *J Biol Chem* 273 11044-11048 (1998)
51. Brookes PS: Mitochondrial nitric oxide synthase. *Mitochondrion* 3, 187-204 (2004)

52. Haynes V, S. Elfering, N. Traaseth & C. Giulivi: Mitochondrial nitric-oxide synthase, enzyme expression, characterization, and regulation. *J Bioenerg Biomembr* 36, 341-346 (2004)
53. Ghafourifar P & E. Cadenas: Mitochondrial nitric oxide synthase. *Trends Pharmacol Sci* 26, 190-195 (2005)
54. Traaseth N, S. Elfering, J. Solien, V. Haynes & C. Giulivi: Role of calcium signaling in the activation of mitochondrial nitric oxide synthase and citric acid cycle. *Biochim Biophys Acta* 1658, 64-71 (2004)
55. Trimmer BA, J.R. Aprille, D.M. Dudzinski, C.J. Lagace, S.M. Lewis, T. Michel, S. Qazi & R.M. Zayas: Nitric oxide and the control of firefly flashing. *Science* 292, 2486-2488 (2001)
56. Nisoli E, E. Clementi, C. Paolucci, V. Cozzi, C. Tonello, C. Sciorati, R. Bracale, A. Valerio, M. Francolini, S. Moncada & M.O. Carruba: Mitochondrial biogenesis in mammals, the role of endogenous nitric oxide. *Science* 299, 896-899 (2003)
57. Clementi E, G.C. Brown, M. Feelisch & S. Moncada: Persistent inhibition of cell respiration by nitric oxide, crucial role of S-nitrosylation of mitochondrial complex I and protective action of glutathione. *Proc Natl Acad Sci U S A* 95, 7631-7636 (1998)
58. Mannick JB, C. Schonhoff, N. Papeta, P. Ghafourifar, M. Szibor, K. Fang & B. Gaston: S-Nitrosylation of mitochondrial caspases. *J Cell Biol* 154, 1111-1116 (2001)
59. Ghafourifar P, U. Schenk, S.D. Klein & C. Richter: Mitochondrial nitric-oxide synthase stimulation causes cytochrome c release from isolated mitochondria. Evidence for intramitochondrial peroxynitrite formation. *J Biol Chem* 274, 31185-31188 (1999)
60. Poderoso JJ, C. Lisdero, F. Schopfer, N. Riobo, M.C. Carreras, E. Cadenas & A. Boveris: The regulation of mitochondrial oxygen uptake by redox reactions involving nitric oxide and ubiquinol. *J Biol Chem* 274, 37709-37716 (1999)
61. Cadenas E, J.J. Poderoso, F. Antunes & A. Boveris: Analysis of the Pathways of Nitric Oxide Utilization in Mitochondria. *Free Radic Res* 33, 747-756 (2001)
62. Boveris A, S. Alvarez & A. Navarro: The role of mitochondrial nitric oxide synthase in inflammation and septic shock. *Free Radic Biol Med* 33, 1186-1193 (2002)
63. Radi R, A. Cassina, R. Hodara, C. Quijano & L. Castro: Peroxynitrite reactions and formation in mitochondria. *Free Radic Biol Med* 33, 1451-1464 (2002)
64. Bonini MG, R. Radi, G. Ferrer-Sueta, A. M. Ferreira & O. Augusto: Direct EPR detection of the carbonate radical anion produced from peroxynitrite and carbon dioxide. *J Biol Chem* 274, 10802-10806 (1999)
65. Bindoli A: Lipid peroxidation in mitochondria. *Free Radic Biol Med* 5, 247-261 (1988)
66. Richter C, V. Gogvadze, R. Laffranchi, R. Schlapbach, M. Schweizer, M. Suter, P. Walter & M. Yaffee: Oxidants in mitochondria, from physiology to diseases. *Biochim Biophys Acta* 1271, 67-74 (1995)
67. Lenaz G: Role of mitochondria in oxidative stress and ageing. *Biochim Biophys Acta* 1366, 53-67 (1998)
68. Cadenas E & K.J. Davies: Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic Biol Med* 29, 222-230 (2000)
69. Tyler DD: A protective function of superoxide dismutase during respiratory chain activity. *Biochim Biophys Acta* 396, 335-346 (1975)
70. Schild L, T. Reinheckel, I. Wiswedel & W. Augustin: Short-term impairment of energy production in isolated rat liver mitochondria by hypoxia/reoxygenation, involvement of oxidative protein modification. *Biochem J* 328, 205-210 (1997)
71. Zoratti M & I. Szabo: The mitochondrial permeability transition. *Biochim Biophys Acta* 1241, 139-176 (1995)
72. Kowaltowski AJ, R.F. Castilho & A.E. Vercesi: Mitochondrial permeability transition and oxidative stress. *FEBS Lett* 495, 12-15 (2001)
73. Lemasters JJ, A.L. Nieminen, T. Qian, L.C. Trost, S.P. Elmore, Y. Nishimura, R.A. Crowe, W.E. Cascio, C.A. Bradham, D.A. Brenner & B. Herman: The mitochondrial permeability transition in cell death, a common mechanism in necrosis, apoptosis and autophagy. *Biochim Biophys Acta* 1366, 177-196 (1998)
74. Crompton M: The mitochondrial permeability transition pore and its role in cell death. *Biochem J* 341, 233-249 (1999)
75. Green DR & J. C. Reed: Mitochondria and apoptosis. *Science* 281, 1309-1312 (1998)
76. He L & J.J. Lemasters: Regulated and unregulated mitochondrial permeability transition pores, a new paradigm of pore structure and function? *FEBS Lett* 512, 1-7 (2002)
77. Beutner G, A. Ruck, B. Riede & D. Brdiczka: Complexes between porin, hexokinase, mitochondrial creatine kinase and adenylate translocator display properties of the permeability transition pore. Implication for regulation of permeability transition by the kinases. *Biochim Biophys Acta* 1368, 7-18 (1998)
78. Brdiczka D, G. Beutner, A. Ruck, M. Dolder & T. Wallimann: The molecular structure of mitochondrial contact sites. Their role in regulation of energy metabolism and permeability transition. *Biofactors* 8, 235-242 (1998)
79. Halestrap AP, G.P. McStay & S.J. Clarke: The permeability transition pore complex, another view. *Biochimie* 84, 153-166 (2002)
80. Fagian MM, L. Pereira-da-Silva, I.S. Martins & A.E. Vercesi: Membrane protein thiol cross-linking associated with the permeabilization of the inner mitochondrial membrane by Ca^{2+} plus prooxidants. *J Biol Chem* 265, 19955-19960 (1990)
81. Siliprandi D, G. Scutari, F. Zoccarato & N. Siliprandi: Action of 'diamide' on some energy linked processes of rat liver mitochondria. *FEBS Lett* 42, 197-199 (1974)
82. Lenartowicz E, P. Bernardi & G.F. Azzone: Phenylarsine oxide induces the cyclosporin A-sensitive membrane permeability transition in rat liver mitochondria. *J Bioenerg Biomembr* 23, 679-688 (1991)
83. Valle VG, M.M. Fagian, L.S. Parentoni, A.R. Meinicke & A.E. Vercesi: The participation of reactive oxygen species and protein thiols in the mechanism of mitochondrial inner membrane permeabilization by calcium plus prooxidants. *Arch Biochem Biophys* 307, 1-7 (1993)
84. Castilho RF, A.J. Kowaltowski, A.R. Meinicke, E.J. Bechara & A.E. Vercesi: Permeabilization of the inner mitochondrial membrane by Ca^{2+} ions is stimulated by t-

butyl hydroperoxide and mediated by reactive oxygen species generated by mitochondria. *Free Radic Biol Med* 18, 479-486 (1995)
85. Kowaltowski AJ, R.F. Castilho, M.T. Grijalba, E.J. Bechara & A.E. Vercesi: Effect of inorganic phosphate concentration on the nature of inner mitochondrial membrane alterations mediated by Ca^{2+} ions. A proposed model for phosphate-stimulated lipid peroxidation. *J Biol Chem* 271, 2929-2934 (1996)
86. Kowaltowski AJ, R.F. Castilho & A.E. Vercesi: Opening of the mitochondrial permeability transition pore by uncoupling or inorganic phosphate in the presence of Ca^{2+} is dependent on mitochondrial-generated reactive oxygen species. *FEBS Lett* 378, 150-152 (1996)
87. Frei B, K.H. Winterhalter & C. Richter: Mechanism of alloxan-induced calcium release from rat liver mitochondria. *J Biol Chem* 260, 7394-7401 (1985)
88. Hermes-Lima M, V.G. Valle, A.E. Vercesi & E.J. Bechara: Damage to rat liver mitochondria promoted by delta-aminolevulinic acid-generated reactive oxygen species, connections with acute intermittent porphyria and lead-poisoning. *Biochim Biophys Acta* 1056, 57-63 (1991)
89. Packer MA & M.P. Murphy: Peroxynitrite formed by simultaneous nitric oxide and superoxide generation causes cyclosporin-A-sensitive mitochondrial calcium efflux and depolarisation. *Eur J Biochem* 234, 231-239 (1995)
90. Vercesi AE, A.J. Kowaltowski, M.T. Grijalba, A.R. Meinicke & R.F. Castilho: The role of reactive oxygen species in mitochondrial permeability transition. *Biosci Rep* 17, 43-52 (1997)
91. Grijalba MT, A.E. Vercesi & S. Schreier: Ca^{2+}-induced increased lipid packing and domain formation in submitochondrial particles. A possible early step in the mechanism of Ca^{2+}-stimulated generation of reactive oxygen species by the respiratory chain. *Biochemistry* 38, 13279-13287 (1999)
92. Bernardi P, S. Vassanelli, P. Veronese, R. Colonna, I. Szabo & M. Zoratti: Modulation of the mitochondrial permeability transition pore. Effect of protons and divalent cations. *J Biol Chem* 267, 2934-2939 (1992)
93. Kowaltowski AJ, A.E. Vercesi & R.F. Castilho: Mitochondrial membrane protein thiol reactivity with N-ethylmaleimide or mersalyl is modified by Ca^{2+}, correlation with mitochondrial permeability transition. *Biochim Biophys Acta* 1318, 395-402 (1997)
94. Kowaltowski AJ & R.F. Castilho: Ca^{2+} acting at the external side of the inner mitochondrial membrane can stimulate mitochondrial permeability transition induced by phenylarsine oxide. *Biochim Biophys Acta* 1322, 221-229 (1997)
95. Kroemer G, B. Dallaporta & M. Resche-Rigon: The mitochondrial death/life regulator in apoptosis and necrosis. *Annu Rev Physiol* 60, 619-642 (1998)
96. Fiers W, R. Beyaert, W. Declercq & P. Vandenabeele: More than one way to die, apoptosis, necrosis and reactive oxygen damage. *Oncogene* 18, 7719-7730 (1999)
97. Liu X, C.N. Kim, J. Yang, R. Jemmerson and X. Wang: Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. *Cell* 86, 147-157 (1996)
98. Susin SA, H.K. Lorenzo, N. Zamzami, I. Marzo, B.E. Snow, G.M. Brothers, J. Mangion, E. Jacotot, P. Costantini, M. Loeffler, N. Larochette, D.R. Goodlett, R. Aebersold, D.P. Siderovski, J.M. Penninger & G. Kroemer: Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature* 397, 441-446 (1999)
99. Zhivotovsky B, A. Samali, A. Gahm & S. Orrenius: Caspases: their intracellular localization and translocation during apoptosis. *Cell Death Differ.* 6, 644-651 (1999).
100. Wajant H: The Fas signaling pathway, more than a paradigm. *Science* 296, 1635-1636 (2002)
101. Kelekar A & C.B. Thompson: Bcl-2-family proteins, the role of the BH3 domain in apoptosis. *Trends Cell Biol* 8, 324-330 (1998)
102. Reed JC: Bcl-2 family proteins. *Oncogene* 17, 3225-3236 (1998)
103. Cory S, D.C. Huang & J.M. Adams: The Bcl-2 family, roles in cell survival and oncogenesis. *Oncogene* 22, 8590-8607 (2003)
104. Leist M, B. Single, A.F. Castoldi, S. Kuhnle & P. Nicotera: Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. *J Exp Med* 185, 1481-1486 (1997)
105. Halestrap AP, S.J. Clarke & S.A. Javadov: Mitochondrial permeability transition pore opening during myocardial reperfusion-a target for cardioprotection. *Cardiovasc Res* 61, 372-385 (2004)
106. Cuzzocrea S, D.P. Riley, A.P. Caputi & D. Salvemini: Antioxidant therapy, a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. *Pharmacol Rev* 53, 135-159 (2001)
107. Murry CE, R.B. Jennings & K.A. Reimer: Preconditioning with ischemia, a delay of lethal cell injury in ischemic myocardium. *Circulation* 74, 1124-1136 (1986)
108. Vanden Hoek TL, L.B. Becker, Z. Shao, C. Li & P.T. Schumacker: Reactive oxygen species released from mitochondria during brief hypoxia induce preconditioning in cardiomyocytes. *J Biol Chem* 273, 18092-18098 (1998)
109. Xu M, Y. Wang, K. Hirai, A. Ayub & M. Ashraf: Calcium preconditioning inhibits mitochondrial permeability transition and apoptosis. *Am J Physiol Heart Circ Physiol* 280, H899-908 (2001)
110. Lawson CS & J.M. Downey: Preconditioning, state of the art myocardial protection. *Cardiovasc Res* 27, 542-550 (1993)
111. Parratt JR: Protection of the heart by ischaemic preconditioning, mechanisms and possibilities for pharmacological exploitation. *Trends Pharmacol Sci* 15, 19-25 (1994)
112. Facundo HT, M. Fornazari & A.J. Kowaltowski: Tissue protection mediated by mitochondrial K+ channels. *Biochim Biophys Acta* 1762, 202-212 (2006)
113. Murata M, M. Akao, B. O'Rourke & E. Marban: Mitochondrial ATP-sensitive potassium channels attenuate matrix Ca2+ overload during simulated ischemia and reperfusion, possible mechanism of cardioprotection. *Circ Res* 89, 891-898 (2001)
114. Hausenloy DJ, H.L. Maddock, G.F. Baxter & D.M. Yellon: Inhibiting mitochondrial permeability transition pore opening, a new paradigm for myocardial preconditioning? *Cardiovasc Res* 55 534-543 (2002)
115. Javadov SA, S. Clarke, M. Das, E.J. Griffiths, K.H. Lim & A.P. Halestrap: Ischaemic preconditioning inhibits

opening of mitochondrial permeability transition pores in the reperfused rat heart. *J Physiol* 549, 513-524 (2003)
116. Facundo HT, J.G. de Paula & A.J. Kowaltowski: Mitochondrial ATP-sensitive K^+ channels prevent oxidative stress, permeability transition and cell death. *J Bioenerg Biomembr*. 37, 75-82 (2005)
117. Garlid KD, P. Dos Santos, Z.J. Xie, A.D. Costa & P. Paucek: Mitochondrial potassium transport, the role of the mitochondrial ATP-sensitive K^+ channel in cardiac function and cardioprotection. *Biochim Biophys Acta* 1606, 1-21 (2003)
118. Belisle E & A.J. Kowaltowski: Opening of mitochondrial K^+ channels increases ischemic ATP levels by preventing hydrolysis. *J Bioenerg Biomembr* 34, 285-298 (2002)
119. Ferranti R, M.M. da Silva & A.J. Kowaltowski: Mitochondrial ATP-sensitive K^+ channel opening decreases reactive oxygen species generation. *FEBS Lett* 536, 51-55 (2003)
120. Carroll R, V.A. Gant & D.M. Yellon: Mitochondrial K_{ATP} channel opening protects a human atrial-derived cell line by a mechanism involving free radical generation. *Cardiovasc Res* 51, 691-700 (2001)
121. Forbes RA, C. Steenbergen & E. Murphy: Diazoxide-induced cardioprotection requires signaling through a redox-sensitive mechanism. *Circ Res* 88, 802-809 (2001)
122. Facundo HT, R.S. Carreira, J.G. de Paula, C.X. Santos, R. Ferranti, F.R. Laurindo & A.J. Kowaltowski: Ischemic preconditioning requires increases in reactive oxygen release independent of mitochondrial K^+ channel activity. *Free Radic Biol Med* 40, 469-479 (2006)
123. Beal MF, B.T. Hyman & W. Koroshetz: Do defects in mitochondrial energy metabolism underlie the pathology of neurodegenerative diseases? *Trends Neurosci* 16, 125-131 (1993)
124. Coyle JT & P. Puttfarcken: Oxidative stress, glutamate, and neurodegenerative disorders. *Science* 262, 689-695 (1993)
125. Orth M & A.H. Schapira: Mitochondria and degenerative disorders. *Am J Med Genet* 106, 27-36 (2001)
126. Schon EA & G. Manfredi G: Neuronal degeneration and mitochondrial dysfunction. *J Clin Invest* 111, 303-312 (2003)
127. Rothman SM & J.W. Olney: Excitotoxicity and the NMDA receptor. *Trends Neurosci* 10, 299-302 (1987)
128. Fiskum G, A.N. Murphy & M.F. Beal: Mitochondria in neurodegeneration: acute ischemia and chronic neurodegenerative diseases. *J Cereb Blood Flow Metab* 19, 351-369 (1999)
129. Choi DW: Ionic dependence of glutamate neurotoxicity. *J Neurosci* 7, 369-379 (1987)
130. Nicholls DG & S.L. Budd: Mitochondria and neuronal survival. *Physiol Rev* 80, 315-360 (2000)
131. Friberg H, M. Ferrand-Drake, F. Bengtsson, A.P. Halestrap & T. Wieloch: Cyclosporin A, but not FK 506, protects mitochondria and neurons against hypoglycemic damage and implicates the mitochondrial permeability transition in cell death. *J Neurosci* 18, 5151-5159 (1998)
132. Matsumoto S, H. Friberg, M. Ferrand-Drake & T. Wieloch: Blockade of the mitochondrial permeability transition pore diminishes infarct size in the rat after transient middle cerebral artery occlusion. *J Cereb Blood Flow Metab* 19, 736-741 (1999)
133. Castilho RF, O. Hansson, M.W. Ward, S.L. Budd & D.G. Nicholls: Mitochondrial control of acute glutamate excitotoxicity in cultured cerebellar granule cells. *J Neurosci* 18, 10277-10286 (1998)
134. Brustovetsky N & J.M. Dubinsky: Limitations of cyclosporin A inhibition of the permeability transition in CNS mitochondria. *J Neurosci* 20, 8229-8237 (2000)
135. Alano CC, G. Beutner, R.T. Dirksen, R.A. Gross & S.S. Sheu: Mitochondrial permeability transition and calcium dynamics in striatal neurons upon intense NMDA receptor activation. *J Neurochem* 80, 531-538 (2002)
136. Maciel EN, G.S. Kaminski Schierle, O. Hansson, P. Brundin & R.F. Castilho: Cyclosporin A and Bcl-2 do not inhibit quinolinic acid-induced striatal excitotoxicity in rodents. *Exp Neurol* 183, 430-437 (2003)
137. Nicholls DG & I.D. Scott: The regulation of brain mitochondrial calcium-ion transport. The role of ATP in the discrimination between kinetic and membrane-potential-dependent calcium-ion efflux mechanisms. *Biochem J* 186, 833-839 (1980)
138. Maciel EN, A.J. Kowaltowski, F.D. Schwalm, J.M. Rodrigues, D.O. Souza, A.E. Vercesi, M. Wajner & R.F. Castilho: Mitochondrial permeability transition in neuronal damage promoted by Ca^{2+} and respiratory chain complex II inhibition. *J Neurochem* 90, 1025-1035 (2004)
139. Lipton P: Ischemic cell death in brain neurons. *Physiol Rev* 79, 1431-1568 (1999)
140. Friberg H, T. Wieloch & R.F. Castilho: Mitochondrial oxidative stress after global brain ischemia in rats. *Neurosci Lett* 334, 111-114 (2002)
141. Mattiasson G, M. Shamloo, G. Gido, K. Mathi, G. Tomasevic, S. Yi, C.H. Warden, R.F. Castilho, T. Melcher, M. Gonzalez-Zulueta, K. Nikolich & T. Wieloch: Uncoupling protein-2 prevents neuronal death and diminishes brain dysfunction after stroke and brain trauma. *Nat Med* 9, 1062-1068 (2003)
142. Langston JW, P. Ballard, J.W. Tetrud & I. Irwin: Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* 219, 979-980 (1983)
143. Betarbet R, T.B. Sherer, G. MacKenzie, M. Garcia-Osuna, A.V. Panov & J.T. Greenamyre: Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci* 3, 1301-1306 (2000)
144. Votyakova TV & I.J. Reynolds: Ca^{2+}-induced permeabilization promotes free radical release from rat brain mitochondria with partially inhibited complex I. *J Neurochem* 93, 526-537 (2005)
145. Sousa SC, E.N. Maciel, A.E. Vercesi & R.F. Castilho: Ca^{2+}-induced oxidative stress in brain mitochondria treated with the respiratory chain inhibitor rotenone. *FEBS Lett* 543, 179-183 (2003)
146. Sousa SC & R.F. Castilho: Protective effect of melatonin on rotenone plus Ca^{2+}-induced mitochondrial oxidative stress and PC12 cell death. *Antioxid Redox Signal* 7, 1110-1116 (2005)
147. Dutra JC, C.S. Dutra-Filho, S.E. Cardozo, C.M. Wannmacher, J.J. Sarkis & M. Wajner: Inhibition of succinate dehydrogenase and beta-hydroxybutyrate dehydrogenase activities by methylmalonate in brain and

liver of developing rats. *J Inherit Metab Dis* 16, 147-153 (1993)
148. Okun JG, F. Horster, L.M. Farkas, P. Feyh, A. Hinz, S. Sauer, G.F. Hoffmann, K. Unsicker, E. Mayatepek & S. Kolker: Neurodegeneration in methylmalonic aciduria involves inhibition of complex II and the tricarboxylic acid cycle, and synergistically acting excitotoxicity. *J Biol Chem* 277, 14674-14680 (2002)
149. Fenton WA, R.A. Gravel & D.S. Rosenblatt: Disorders of propionate and methylmalonate metabolism, In: *The Metabolic and Molecular Basis of Inherited Disease* (C.R. Scriver, A.L. Beaudet, A.D. Valle, W.S. Sly, eds). 8th edition, McGraw-Hill, New York. 94, 2165-2193 (2001)
150. Libby P: The forgotten majority: unfinished business in cardiovascular risk reduction. J Am Coll Cardiol 46, 1225-1228 (2005)
151. Gong DW, Y. He, M. Karas & M. Reitman: Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, beta3-adrenergic agonists, and leptin. *J Biol Chem* 272, 24129-24132 (1997)
152. Samec S, J. Seydoux & A.G. Dulloo: Post-starvation gene expression of skeletal muscle uncoupling protein 2 and uncoupling protein 3 in response to dietary fat levels and fatty acid composition, a link with insulin resistance. *Diabetes* 48, 436-441 (1999)
153. Nisoli E, M.O. Carruba, C. Tonello, C. Macor, G. Federspil & R. Vettor: Induction of fatty acid translocase/CD36, peroxisome proliferator-activated receptor-gamma2, leptin, uncoupling proteins 2 and 3, and tumor necrosis factor-alpha gene expression in human subcutaneous fat by lipid infusion. *Diabetes* 49, 319-324 (2000)
154. Jezek P & K.D. Garlid: Mammalian mitochondrial uncoupling proteins. *Int J Biochem Cell Biol* 30, 1163-1168 (1998)
155. Brand MD & T.C. Esteves: Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3. *Cell Metab* 2, 85-93 (2005)
156. Negre-Salvayre A, C. Hirtz, G. Carrera, R. Cazenave, M. Troly, R. Salvayre, L. Penicaud & L. Casteilla: A role for uncoupling protein-2 as a regulator of mitochondrial hydrogen peroxide generation. *FASEB J* 11, 809-815 (1997)
157. Bao S, A. Kennedy, B. Wojciechowski, P. Wallace, E. Ganaway & W.T. Garvey: Expression of mRNAs encoding uncoupling proteins in human skeletal muscle: effects of obesity and diabetes. *Diabetes* 47, 1935-1940 (1998)
158. Boss O, E. Bobbioni-Harsch, F. Assimacopoulos-Jeannet, P. Muzzin, R. Munger, J.P. Giacobino & A. Golay: Uncoupling protein-3 expression in skeletal muscle and free fatty acids in obesity. *Lancet* 351, 351 (1998)
159. Hidaka S, H. Yoshimatsu, T. Kakuma, H. Sakino, S. Kondou, R. Hanada, K. Oka, Y. Teshima, M. Kurokawa & T. Sakata: Tissue-specific expression of the uncoupling protein family in streptozotocin-induced diabetic rats. *Proc Soc Exp Biol Med* 224, 172-177 (2000)
160. Alberici LC, H.C.F. Oliveira, E.J.B. Bighetti, E.C. de Faria, G.R. Degaspari, C.T. Souza & A.E. Vercesi: Hypertriglyceridemia increases mitochondrial resting respiration and susceptibility to permeability transition. *J. Bioenerg Biomembr* 35, 451-457 (2003)
161. Oliveira HC, R.G. Cosso, L.C. Alberici, E.N. Maciel, A.G. Salerno, G.G. Dorighello, J.A. Velho, E.C. de Faria & A.E. Vercesi: Oxidative stress in atherosclerosis-prone mouse is due to low antioxidant capacity of mitochondria. *FASEB J* 19, 278-280 (2005)
162. Ito Y, N. Azrolan, A. O'Connell, A. Walsh, & J. L. Breslow: Hypertriglyceridemia as a result of human apo CIII gene expression in transgenic mice. *Science* 249, 790-793 (1990)
163. Reaven GM, C.E. Mondon, Y.D. Chen, & J.L. Breslow: Hypertriglyceridemic mice transgenic for human apolipoprotein CIII gene are neither insulin resistant nor hyperinsulinemic. *J Lipid Res* 35, 820-824 (1994)
164. Amaral MEC, H.C.F. Oliveira, E.M. Carneiro, V. Delghingaro-Augusto, E. Vieira, J.A. Berti & A.C. Boschero. Plasma glucose regulation and insulin secretion in hypertriglyceridemic mice *Horm Metab Res* 34, 21-26 (2002)
165. Chisolm GM & D. Steinberg: The oxidative hypothesis of atherogenesis: an overview. *Free Radic Biol Med* 28, 1815-1826 (2000)
166. Velho JA, H. Okanobo, G.R. Degasperi, M.Y. Matsumoto, L.C. Alberici, R.G. Cosso, H.C. Oliveira & A.E. Vercesi: Statins induce calcium-dependent mitochondrial permeability transition. *Toxicology* 219, 124-132 (2006)
167. Kaneta S, K. Satoh, S. Kano, M. Kanda & K. Ichihara: All hydrophobic HMG-CoA reductase inhibitors induce apoptotic death in rat pulmonary vein endothelial cells. *Atherosclerosis* 170, 237-243 (2003)
168. Kubota T, K. Fujisaki, Y. Itoh, T. Yano, T. Sendo & R. Oishi: Apoptotic injury in cultured human hepatocytes induced by HMG-CoA reductase inhibitors. *Biochem Pharmacol* 67, 2175-2186 (2004)
169. Clark LT: Treating dyslipidemia with statins: the risk-benefit profile. *Am Heart J* 145, 387-396 (2003)

Key Words: Mitochondria, Calcium, Potassium, Free Radicals, Apoptosis, Necrosis, Stroke, Heart Attack, Dyslipidemia, Review

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