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Mechanisms of Impaired Mitochondrial Energy Metabolism in Acute and Chronic Neurodegenerative Disorders

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Abstract

Altered mitochondrial energy metabolism contributes to the pathophysiology of acute brain injury caused by ischemia, trauma, and neurotoxins and by chronic neurodegenerative disorders such as Parkinson's and Huntington's diseases. Although much evidence supports that the electron transport chain dysfunction in these metabolic abnormalities has both genetic and intracellular environmental causes, alternative mechanisms are being explored. These include direct, reversible inhibition of cytochrome oxidase by nitric oxide, release of mitochondrial cytochrome c, oxidative inhibition of mitochondrial matrix dehydrogenases and adenine nucleotide transport, the availability of NAD for dehydrogenase reactions, respiratory uncoupling by activities such as that of the permeability transition pore, and altered mitochondrial structure and intracellular trafficking. This review focuses on the catabolism of neuronal NAD and the release of neuronal mitochondrial NAD as important contributors to metabolic dysfunction. In addition, the relationship between apoptotic signaling cascades and disruption of mitochondrial energy metabolism is considered in light of the fine balance between apoptotic and necrotic neural cell death.

Keywords

PARP-1; nitric oxide; calcium; apoptosis; mitochondrial permeability transition

A large body of evidence indicates that mitochondrial dysfunction plays a critical role in the pathophysiologic mechanisms of both acute and chronic neurodegeneration (Fiskum et al., 1999; Lin and Beal, 2006). The consequences of mitochondrial dysfunction are numerous and include oxidative stress, cellular Ca^{2+} dys-homeostasis, promotion of apoptosis, and metabolic failure. The strongest confirmation of the participation of mitochondrial injury in metabolic failure comes from animal models of acute brain injury (Chang et al., 1992; Kuroda et al., 1996) and metabolic imaging studies performed with head injury patients (Marmarou et al., 2005); however, support also exists for mitochondrial metabolic insufficiency in the pathophysiology of genetic encephalomyopathies (DiMauro and Schon, 2003) and neurodegenerative diseases, for example, Parkinson's disease (Wallace, 2005; Tieu et al., 2003).

There are many possible causes of mitochondrial metabolic impairment, as summarized in Table I. Historically, most attention has been paid to the abnormal expression or functioning of electron transport chain (ETC) components. Currently, much research is focusing on how

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oxidative stress impairs respiration, as this could initiate a vicious cycle of redox-stimulated production of reactive oxygen species (ROS) and further inhibition of normal electron transport until the rate of ATP production falls below that of ATP demand, resulting in metabolic failure. The knowledge that nitric oxide is a competitive inhibitor of O_2 at cytochrome oxidase has generated increasing interest, particularly as it is related to ischemic and traumatic brain injury, in which brain nitric oxide is often elevated and tissue O_2 is often depressed (Brown, 1995). The ETC is also an important target of zinc neurotoxicity (Sharpley and Hirst, 2006), which is thought to be important in ischemic brain damage (Sensi and Jeng, 2004). Another very critical cause of impaired ETC activity is the release of cytochrome c through the outer membrane into the cytosol (Polster et al., 2001). Cytochrome c, which transfers electrons from complex III to complex IV, is a peripheral membrane cationic protein that is loosely associated with the outer side of the inner membrane through its interactions with ETC complexes and cardiolipin, a mitochondria-specific phospholipid. As discussed later, release of cytochrome c from the intermembrane space through an osmotically ruptured outer membrane or through proteinaceous or lipidic megapores shifts equilibrium toward the unbound state, therefore inhibiting cellular respiration.

In addition to occurring with impaired ETC activity, mitochondrial dysfunction can occur in response to inhibition of any enzyme, transport, or pore activity necessary for oxidative phosphorylation. Thus, oxidative inactivation of mitochondrial matrix enzymes, for example, pyruvate and α-ketoglutarate dehydrogenases and aconitase, have been implicated in metabolic failure (Tretter and Adam-Vizi, 2000). Evidence also suggests that mitochondrial oxidative stress impairs the adenine nucleotide translocase, necessary for influx of ADP and efflux of ATP (Vesce et al., 2005). These and other metabolites (e.g., respiratory substrates) must traverse the outer membrane through voltage-gated anion channels, also known as mitochondrial porins. Some evidence suggests that under specific conditions, this important pore can exist in a closed state, possibly limiting aerobic energy production (Vander Heiden et al., 2001). In addition to investigations of membrane permeability potentially limiting the availability of substrates for mitochondrial reactions, much interest is currently focused on the availability of the metabolic cofactor NAD. Metabolites and cofactors such as NAD are lost from the matrix following activation of the mitochondrial inner membrane permeability transition, as described in the following section. Moreover, many cytosolic and possibly mitochondrial reactions appear to consume NAD and lower its concentration to a metabolically critical level under certain pathological conditions, particularly those associated with oxidative stress (Ying, 2006). Finally, the last section of this review summarizes the interactions between various apoptotic signaling events and mitochondrial energy metabolism, including the relatively recent discovery that mitochondrial fission and the altered intracellular trafficking of this organelle are closely associated with both apoptosis and metabolic failure (Yuan et al., 2007).

INNER-MEMBRANE PERMEABILITY TRANSITION

One of the most potentially important mechanisms of mitochondrial dysfunction that can result in metabolic failure is prolonged activation of the mitochondrial permeability transition (MPT) pore, an inner-membrane channel that is permeable to solutes with a molecular mass of less than approximately 1,500 Da (for a review, see Zoratti and Szabo, 1995; Bernardi et al., 1999, 2001; Crompton et al., 2002; Halestrap et al., 2002). As a result of this pore opening, the mitochondrial electrochemical hydrogen ion gradient dissipates, the matrix is depleted of pyridine nucleotides, and mitochondria (at least in vitro) swell because of the osmotic uptake of water. Consequently, the mitochondria not only become incapable of ATP synthesis but actively consume ATP, leading to loss of metabolic integrity and cell death.

Calcium is the most important factor that triggers MPT pore opening; however, the probability of opening is modulated by several factors and drugs (Gunter and Pfeiffer, 1990). Adenine nucleotides (ATP, ADP), magnesium (Mg^{2+}), low intramitochondrial pH, and high membrane potential inhibit pore opening (Bernardi et al., 1992, 1999). Conversely, high inorganic phosphate (P_i), oxidative stress, and oxidized pyridine nucleotides promote pore opening (Bernardi et al., 1999). MPT pore formation and regulation of its activation is complex. Because many positive and negative effectors interactively affect the pore in a cell-selective manner, the degree to which the MPT is involved in metabolic dysfunction and death of specific cells under different pathological conditions in vivo is highly variable.

Involvement of the MPT in cell death cascades associated with acute neurological disorders has been described by several laboratories (Okonkwo and Povlishock, 1999; Dubinsky et al., 1999; Friberg and Wieloch, 2002; Kristal et al., 2004; Kristian, 2004; Sullivan et al., 2005; Albrecht and Norenberg, 2006). The findings that the immunosuppressant compound cyclosporin A (CsA) inhibits MPT and exhibits substantial neuroprotective effects against ischemic and traumatic brain injury provides indirect support for a role of MPT in acute brain damage (Uchino et al., 1995, 2002; Matsumoto et al., 1999; Okonkwo and Povlishock, 1999; Yoshimoto and Siesjo, 1999; Sullivan et al., 2000; Alessandri et al., 2002). CsA inhibits the MPT through its binding to a mitochondrial isoform of cyclophilin, cyclophilin D (Woodfield et al., 1988). However, because CsA also binds to other nonmitochondrial cyclophilin isoforms that regulate many other cellular activities, the neuroprotection observed with CsA cannot be conclusively related to MPT inhibition. Nevertheless, CsA analogues such as *N*-methylvaline-4-CsA that inhibit MPT but have limited nonmitochondrial effects also exhibit neuroprotective efficacy (Friberg and Wieloch 2002; Hansson et al., 2004). Other support for the involvement of the MPT in acute brain injury includes observations by Ouyang et al. (1997) indicating mitochondrial membrane damage following cerebral ischemia. These authors detected a redistribution of the mitochondrial isoform of aspartate aminotransferase from mitochondria to the cytosol during the first hour of reperfusion following transient focal ischemia. Furthermore, it has been shown that there is a significant decrease in mitochondrial NAD content following an ischemic insult (Di Lisa et al., 2001). Glutathione uptake by brain mitochondria following cerebral ischemia has also been reported (Zaidan and Sims, 1996, Zaidan et al., 1999). These results were interpreted as indirect evidence of the MPT because the robust increase in permeability of the mitochondrial inner membrane allowed diffusion of NAD and glutathione between the cytosol and the mitochondrial matrix. In addition, these postischemic changes were inhibited by CsA pretreatment.

The most convincing evidence for the MPT having an integral role in acute brain injury comes from experiments performed with cyclophilin D–deficient mice using a reversible focal ischemia model (Schinzel et al., 2005). These cyclophilin D–knockout mice displayed a dramatic reduction in the size of brain infarcts. In addition, isolated liver and brain mitochondria from the cyclophilin D–null mice exhibit elevated Ca^{2+} uptake associated with resistance to Ca^{2+}-induced swelling. As discussed later, these and other observations strongly support the role of the MPT in acute necrotic neuronal death but minimize the role of the MPT in apoptosis.

PYRIDINE NUCLEOTIDE METABOLISM

Pyridine nucleotides are the most prevalent redox carriers in all organisms. The NAD/NADH ratio (i.e., the redox state) is a powerful regulator of glycolysis, the TCA cycle, and oxidative phosphorylation. Moreover, the content and redox state of both NAD and NADP play major roles in additional cellular functions, including Ca^{2+} homeostasis, gene expression, protection against oxidative stress, and programmed cell death (Ziegler, 2000).

In addition to the many redox reactions that either reduce or oxidize NAD(H), there are many reactions that consume NAD, including those catalyzed by poly (ADP-ribose) polymerases (PARPs), mono-(ADP-ribose) transferases (ARTs), histone deacetylases, and bifunctional ADP-ribosyl cyclases/cyclic ADP-ribose hydrolases (Ying, 2006). Under physiological conditions, the level of NAD in the brain is primarily controlled by the NADase enzyme CD38. Aksoy et al. (2006) found that NADase activity in the plasma membrane, mitochondria, endoplasmic reticulum, and nuclei is absent in the brain of CD38-deficient mice, whose tissue NAD level is 10-fold higher than that in wild-type animals. Under pathological conditions, for example, during cerebral ischemia/reperfusion, oxidative stress, hypoglycemia, ammonia toxicity, and glutamate excitotoxicity, PARP-1 appears to be the most potent NAD-consuming enzyme (Zhang et al., 1994; Szabo and Dawson, 1998; Narasimhan et al., 2003; Suh et al., 2003; Kosenko et al., 2004). PARP-1 becomes highly activated because of its role in facilitating repair of damaged DNA. Activated PARP-1 hydrolyzes NAD and transfers the ADP-ribose moieties to form poly(ADP-ribose) on acceptor proteins (Shall and de Murcia, 2000). This activity can potentially result in a dramatic decline in cellular NAD, particularly under metabolically stressed conditions, in which a decline in cellular ATP can limit NAD biosynthesis via the ATP-dependent NAD synthetase reaction (Fig. 1). Once the NAD concentration falls below the approximately 1 mM level necessary to sustain the glycolytic glyceraldehyde-3P dehydrogenase reaction or the approximately 0.1 mM level necessary for intramitochondrial dehydrogenase reactions, the rate of ATP production is impaired, resulting in a vicious cycle that if not reversed, eventually results in permanent metabolic failure and necrotic cell death (Berger, 1985; Carson et al., 1986). Depletion of NAD can also induce both necrosis and apoptosis by multiple additional mechanisms, including promotion of the mitochondrial permeability transition (MPT) and modulation of NAD-dependent sirtuins, protein deacetylases that regulate cell-death genetic programs (Yeung et al., 2004; Muruganandham et al., 2005; Tanaka et al., 2005).

Most cellular NAD(H) is concentrated in mitochondria, and most of this pool is bound to protein (Shuttleworth et al., 2003; Vishwasrao et al., 2005). Our interest in studying the effects of anoxia and reoxygenation on cellular and mitochondrial NAD(P)H fluorescence in vitro in neurons and astrocytes arose from observations made in vivo demonstrating a decrease in intrinsic NAD(P)H fluorescence on the surface of the cerebral cortex below baseline following global cerebral ischemia and reperfusion (Perez-Pinzon et al., 1998). This decrease in fluorescence and the associated neuronal electrophysiologic abnormalities were ameliorated by the presence of antioxidants (Perez-Pinzon et al., 1997) and by perfusion with normoxic compared to hyperoxic gas mixtures (Feng et al., 1998), suggesting that reactive oxygen species (ROS) mediate the decrease in NAD(P)H fluorescence. Although NAD(P)H hyperoxidation can be a result of many factors, such as simply elevated respiration or impaired production of NADH from enzymes like pyruvate dehydrogenase, the observations of Swanson and colleagues demonstrating catabolism of NAD(H) in oxidative stress paradigms suggested an alternative explanation (Alano et al., 2004; Zeng et al., 2007).

The experiments described in Figure 2 indicate that exposure of primary neuronal cultures to chemical anoxia (cyanide) can result in loss of NAD(P)H fluorescence that is partially irreversible and is at least partially mediated by PARP-1 activity. Figure 2A shows the perfusion of neurons with cyanide in the absence of glucose results in an immediate rise in fluorescence because of inhibition of electron flow from NADH to O_2 through the mitochondrial respiratory chain, followed by a slow decline in fluorescence toward the baseline value. The additional presence of the nitric oxide–generating molecule DETA-NO increased the rate of this decline, indicating that oxidative stress exacerbates this phenomenon. The involvement of PARP-1 in fluorescence decline is indicated by the partial inhibition observed in the presence of the PARP-1 inhibitor 3,4-dihydro-5-[4-(1-piperidinyl)butoxy]-1(2H)-isoquinolinone (DPQ). As the mitochondrial permeability transition has been implicated in

this phenomenon in other stress paradigms, we also tested the effect of cyclosporin A, a permeability transition inhibitor, and found that it also partially inhibited the loss of fluorescence.

Additional experiments were performed to determine if the decline in neuronal pyridine nucleotide fluorescence was a result of a net reduction in maximal fluorescence or of NAD(P)H oxidation. As shown In Figure 2B, when compared to neurons exposed to cyanide and DETA-NO in the absence of glucose, cells exposed to cyanide alone maintained a higher and more stable fluorescence level. Subsequent perfusion with cyanide-free media resulted in a fall in fluorescence beyond the baseline following either condition. A second perfusion with cyanide resulted in a rise in fluorescence similar to that elicited by the initial exposure for cells bathed in glucose. In contrast, for cells exposed to DETA-NO in the absence of glucose, the peak fluorescence obtained with the second exposure to cyanide was substantially lower than that elicited after the first exposure, indicating a net loss in maximal pyridine nucleotide fluorescence.

We recently reported a similar net loss of pyridine nucleotide fluorescence for neurons exposed to severe hypoxia (O_2 deprivation) plus glucose deprivation in the presence of nitric oxide (Kahraman and Fiskum, 2007). Our recent unpublished results from enzymatic measurements of total NAD(H) in cell extracts confirmed that total NAD(H) is depleted during these experiments. Depletion of the total pool of NAD(H) has been reported for experiments performed in vivo using a rat model of cerebral ischemia/reperfusion (Welsh et al., 1982). In addition to consumption of total tissue NAD(H), profound depletion of the mitochondrial NAD(H) pool has been reported after ischemia/reperfusion in other tissues, including the heart and kidney (Di Lisa et al., 2001; Feldkamp et al., 2004). This degradation of mitochondrial NAD(H) was also largely inhibited by cyclosporin A (Di Lisa et al., 2001). Because mitochondrial membranes are not permeable to NAD and NADH, loss of the mitochondrial pool of pyridine nucleotides could be explained by the opening of the MPT pore, which releases NAD and makes it available for NAD glycohydrolase located outside the matrix space or PARP-1 in the cytosol and nucleus (Fig. 3). Another explanation for depletion of the mitochondrial NAD pool could be the presence of mitochondrial PARP that is activated by oxidative damage to mitochondrial DNA (Du et al., 2003). We observed partial protection by both the specific PARP-1 inhibitor DPQ and cyclosporin A against chemical hypoxia–induced decline in NADH fluorescence in cortical neurons (Fig. 2B), suggesting that activation of both MPT and PARP-1 contributes to the depletion of the mitochondrial NAD pool during chemical hypoxia (Fig. 3). Additional work is in progress to determine if the MPT is activated directly by peroxynitrite or if the main cause of activation is not having the glycolytically generated reducing power necessary for avoiding activation of the permeability transition through protein sulfhydryl oxidation.

APOPTOTIC SIGNAL TRANSDUCTION

Mitochondria are central regulators of the intrinsic apoptotic pathway, and in some cells (including neurons), they are also recruited in the extrinsic death receptor (DR) pathway, induced by DR ligands (i.e., TNF-α, FasL). The current understanding of mitochondrial involvement in apoptosis indicates that the key event in this process is the permeabilization of the OMM and the release of several apoptogenic proteins (CytC, AIF, Smac/DIABLO, EndoG, Omi/Htra2) from mitochondrial intermembrane space, leading to cell demise in a caspase-dependent or -independent manner. The impact of apoptotic signaling on mitochondria is now appreciated as being more complex and extending beyond regulation of OMM permeability to include effects on mitochondrial energy metabolism, ROS generation, Ca^{2+} metabolism, the intramitochondrial structure, and the mitochondrial filament network.

The release of apoptogenic proteins from mitochondria can occur through two distinct mechanisms: selective OMM permeabilization by protein or lipidic pores or nonspecific rupture of the OMM. Bcl-2 family proteins, the most important regulators of the intrinsic pathway, function primarily to control OMM permeability, although other mitochondrial and extramitochondrial mechanisms (i.e., ER-related) have also been described (Walter and Hajnoczky, 2005). Selective permeabilization of OMM is triggered by activated BH3-only proteins (e.g., Bid, Bim, Bad, Noxa, and Puma) that induce oligomerization of multidomain proapoptotic Bax/Bak and OMM pore formation and is antagonized by Bcl-2-like antiapoptotic members. Reconstitution experiments demonstrated that Bax/Bak and a BH3-only protein (e.g., tBid)/BH3 domain peptide) are the minimal components required. Contrary to early observations, this process occurs without loss of IMM integrity or MPT induction (Polster et al., 2001; Kuwana et al., 2002). Involvement of other OMM proteins (e.g., VDAC) has also been suggested, although their requirement for Bax/BH3-only induced protein release has been questioned (Rostovtseva et al., 2005). However, regulation of VDAC by antiapoptotic Bcl-2 proteins could affect apoptosis by modulating metabolite flux though OMM (Vander Heiden et al., 2001).

Another mechanism of release of apoptogenic proteins involves nonselective rupture of the OMM, following loss of IMM permeability and osmotic swelling of mitochondria, such as that following MPT induction. The MPT initially was proposed as a universal mechanism for mitochondrial involvement in apoptosis and release of apoptogenic proteins (Zamzami and Kroemer, 2001). Although hallmarks of the MPT [loss of IMM potential ($\Delta\Psi$) and swelling of mitochondria] are observed in some cells during apoptosis, and pharmacologic inhibition of MPT protects against apoptosis in some cases, the role of the MPT in apoptosis has been controversial. Recent studies using cells from mice deficient in the cyclophilin D (CyD) component of the PT pore demonstrated that MPT is not required for apoptosis but is involved in necrotic death in response to oxidative stress (Baines et al., 2005; Basso et al., 2005; Nakagawa et al., 2005). The importance of this pathway for acute brain injury is highlighted by the fact that animals deficient in CyD display a marked reduction in infarct size after middle cerebral artery occlusion (Schinzel et al., 2005). MPT and mitochondrial dysfunction can still be promoted by apoptotic signaling (including proapoptotic Bcl-2 proteins), and such effects could contribute to activation of alternate death pathways (e.g., necrotic, autophagic) under conditions in which execution of the apoptotic program is blocked (i.e., deficiency of downstream caspases, ATP depletion).

Bcl-2 and Bax are known to exert opposing effects on the releasable pool of ER Ca^{2+} and could therefore indirectly modulate Ca^{2+}-induced MPT induction following injury (Breckenridge et al., 2003). Like Bcl-2, the Bax-inhibitor-1 (BI-1) protein, functioning in the ER, also reduces the releasable pool of Ca^{2+}. Its overexpression inhibits neuronal death following oxygen glucose deprivation, and *bi-1*$^{-/-}$ mice have increased tissue loss following stroke (Chae et al., 2004; Dohm et al., 2006). Mechanisms distinct from Bax/Bak activation or neutralization of antiapoptotic Bcl-2 proteins appear to mediate mitochondrial dysfunction triggered by some BH3-only proteins. Bnip3 induces a caspase-independent and CsA-sensitive necrotic type of death, and unlike other BH3-only proteins, Bnip3 can also induce autophagic death (Vande Velde et al., 2000). Noxa, a BH3-only derepressor that binds Mcl-1, can induce death through a pathway requiring a ROS increase upstream of caspase activation. Unlike tBid, Noxa-induced CytC release from isolated mitochondria is CsA dependent (Seo et al., 2003; Kim et al., 2004a). Both Noxa and Bnip3 are upregulated in the brain following ischemia (Kim et al., 2004a; Schmidt-Kastner et al., 2004). Among non-Bcl-2 proteins, a recently identified protein, Apop-1, localizes to mitochondria and induces apoptosis and caspase activation through a CypD-dependent but Bcl-2/Bax-independent pathway, suggesting that Apop-1 directly targets the PT pore component CypD (Yasuda et al., 2006). Although Apop-1 appears to be highly expressed in the brain, its potential role in acute brain injury is currently unknown.

Another event associated with induction of apoptosis is the decrease in mitochondrial membrane potential ($\Delta\psi$) that at least in some cases reflects impairment of mitochondrial respiration. One consequence of OMM permeabilization and CytC redistribution (in addition to caspase-9 and -3 activation) is subsequent inhibition of oxidative phosphorylation, a decrease in$\Delta\Psi$, and increased generation of ROS (Starkov et al., 2002). Mitochondrial energy metabolism can also be affected by downstream steps in the apoptotic cascade through caspase-mediated cleavage of the p75 subunit (75 kDa) of complex I, eventually leading to impairment of respiration and increased ROS formation (Ricci et al., 2004). Like CytC, other apoptogenic factors released from mitochondria (i.e., AIF, HtrA2/Omi) also have normal physiologic roles in their mitochondrial location. The absence of functional HtrA2/Omi promotes increased sensitivity of neurons to oxidative stress and MPT induction, and a deficiency in AIF has similar effects (reviewed in Lindholm et al., 2004).

Mitochondria form a dynamic tubular network whose structure is regulated by fusion and fission events and mitochondrial movements, presumably coordinating mitochondrial activity with local changes in cellular metabolism. In addition to OMM permeabilization, profound changes in the intramitochondrial structure, involving the reorganization of mitochondrial cristae and alterations of the mitochondrial tubular network, occur during the early phases of apoptotic death. Alterations in mitochondrial structure have also been linked with regulation of mitochondrial metabolism (Youle and Karbowski, 2005; Cereghetti and Scorrano, 2006; Mannella, 2006; McBride et al., 2006). Members of the Bcl-2 family have recently been implicated in regulation of fusion/fission events in apoptosis in both *C. elegans* and mammalian cells (Delivani et al., 2006). Proapoptotic Bax has been proposed to cooperate with components of the fusion/fission machinery [Drp1, mitofusin 2 (Mfn2)] to induce mitochondrial fragmentation during apoptosis. Mitochondrial fission has also been implicated in the process of CytC release (reviewed in Youle and Karbowski, 2005; Parone and Martinou, 2006). In apoptosis, a dramatic remodeling of the intramitochondrial structure occurs almost concurrently with fragmentation and CytC release. Scorrano et al. (2002) found that in addition to inducing Bax/Bak oligomerization in the OMM, the proapoptotic protein tBid also induces the distinct process of remodeling of the IMM and disruption of the mitochondrial cristae junctions, resulting in maximal CytC release. This activity of tBid appears to involve BH3-independent interactions with cardiolipin at the mitochondrial contact sites (Kim et al., 2004b). The BH3-only protein BIK was also recently shown to regulate an ER pathway inducing mitochondrial fragmentation and Drp1-dependent remodeling and opening of cristae, independent of OMM permeabilization (Germain et al., 2005). The fusion-related dynamin-like GTPase, OPA1, and the IMM integral protease PARL appear to regulate the maintenance of the closed conformation of cristae. OPA1 deficiency induces changes in cristae morphology reminiscent of those induced by tBid and sensitizes cells to apoptosis and CytC release (reviewed in Cereghetti and Scorrano, 2006).

The mitochondrial fission/fusion machinery and the dynamic reorganization of cristae have also been implicated as important regulators of mitochondrial metabolism (Mannella, 2006). Fragmented and fusion-incompetent mitochondria in Opa1- or mitofusin-deficient cells exhibit greatly reduced O_2 consumption and electrochemical potential. Although loss of fusion machinery results in reduced mitochondrial metabolism, overexpression of Mfn2 leads to an increase in respiratory complexes, oxidative phosphorylation, and glucose utilization by cells. The link between mitochondrial morphology and metabolism is further strengthened by observations that hyperglycemia-induced elevated production of ROS requires dynamic changes in mitochondrial morphology and that inhibition of mitochondrial fission prevents an increase in ROS in these conditions (McBride et al., 2006; Yu et al., 2006). Recent studies indicate that mitochondrial fission and impaired mitochondrial movement, events detected early during ischemia or anoxia (Solenski et al., 2002; Zanelli et al., 2006), could mediate NO neurotoxicity. Nitric oxide induces profound fission and ultrastructural mitochondrial changes

in cortical neurons, which are inhibited by Mfn1 (Barsoum et al., 2006). These changes occur well before cell death and are associated with decreased ATP levels, increased free-radical generation, and autophagy (Barsoum et al., 2006).

Such structural and functional mitochondrial alterations induced in injured neurons concurrently with activation of the apoptotic cascade are likely to exert an important influence on cell death, with different cell types showing different susceptibilities (e.g., astrocytes vs. neurons) and could be especially critical for cells with high energy demand such as neurons; in addition, they could also shift cell death to nonapoptotic pathways in cells in which there is a relative deficit (i.e., mature neurons) of downstream apoptotic effectors (e.g., caspase-3, Apaf-1).

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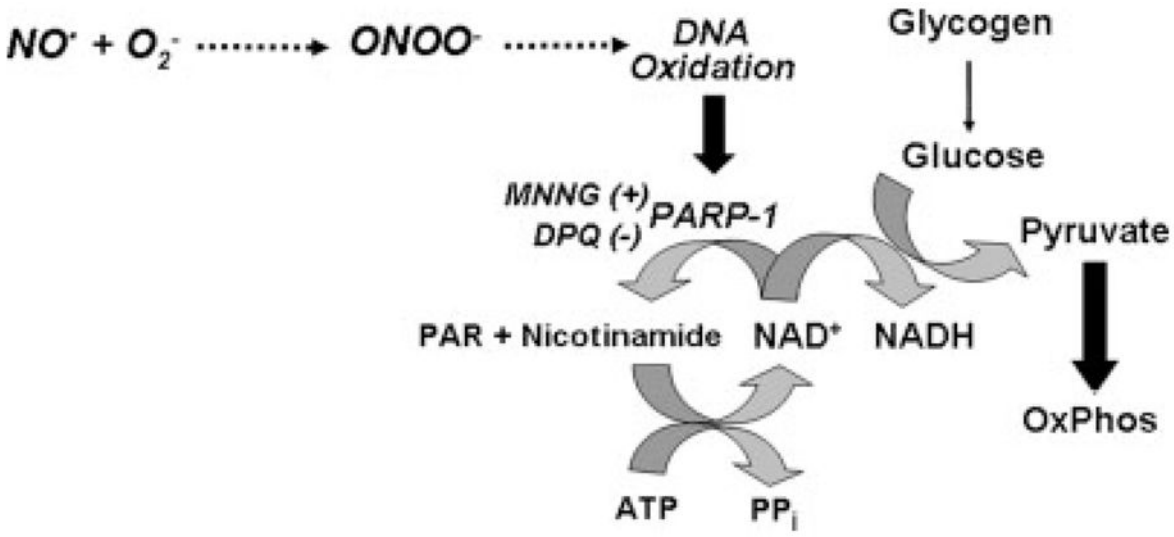

Fig. 1.

Oxidative stress–initiated metabolism of cytosolic NAD by PARP-1. Superoxide (O_2^-), produced from mitochondria and other sources, reacts with nitric oxide (NO·), producing peroxynitrite ($ONOO^-$), a powerful oxidant of DNA and proteins. DNA oxidation activates poly(ADP-ribose) polymerase 1 (PARP-1), which metabolizes NAD to nicotinamide and poly (ADP-ribosylated) (PAR) proteins. The fall in NAD can inhibit the glycolytic production of pyruvate, a major source of fuel for oxidative phosphorylation (OxPhos). Resynthesis of NAD from nicotinamide is dependent on the energy supplied by ATP. PARP-1 can be alternatively activated by exposing cells to MNNG and inhibited by exposure to DPQ.

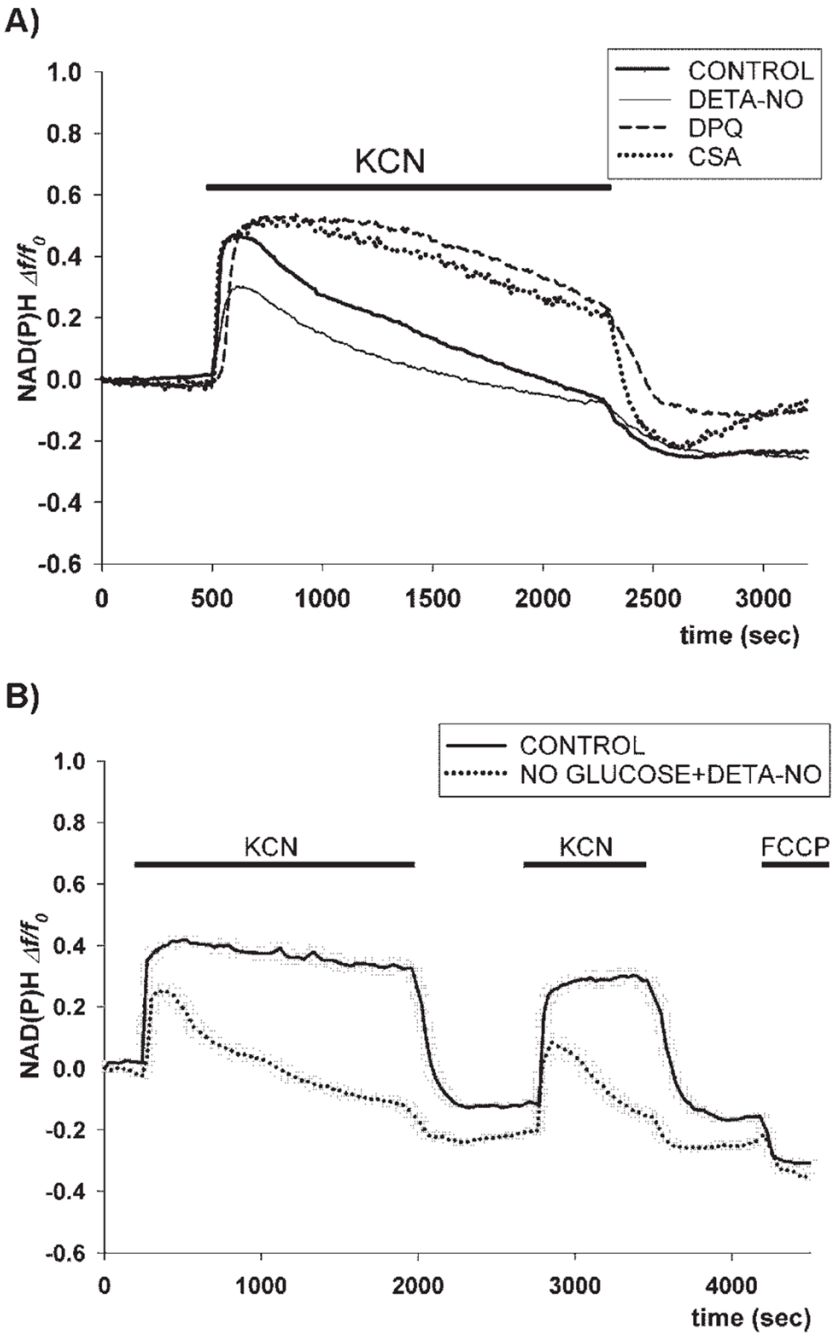

Fig. 2.

Fluorescence microscopy measurement of changes in neuronal NAD(P)H induced by exposure to cyanide. Primary cultures of rat cortical neurons were prepared and maintained on coverslips for 10–14 days prior to fluorescent measurement of NAD(P)H fluorescence, as described in detail in Kahraman and Fiskum (2007). **A:** Cyanide-induced loss of NAD(P)H fluorescence in the absence of glucose promoted by DETA-NO and partially inhibited by the PARP-1 inhibitor 3,4-dihydro-5-[4-(1-piperidinyl)butoxy]-1(2H)-isoquinolinone (DPQ) and by the permeability transition inhibitor cyclosporin A (CSA). **B:** Loss of NAD(P)H fluorescence induced by KCN in the absence of glucose and the presence of DETA-NO partially irreversible after washing out the KCN and subsequent KCN exposure. Fluorescence in the presence of

the respiratory uncoupler FCCP represents maximal NAD(P)H oxidation. Cells were superfused with artificial CSF in the absence or the presence of 15 mM glucose, 1 mM KCN, 5 μM FCCP, 200 μM DETA-NO, 30 μM DPQ, and 1 μM CSA.

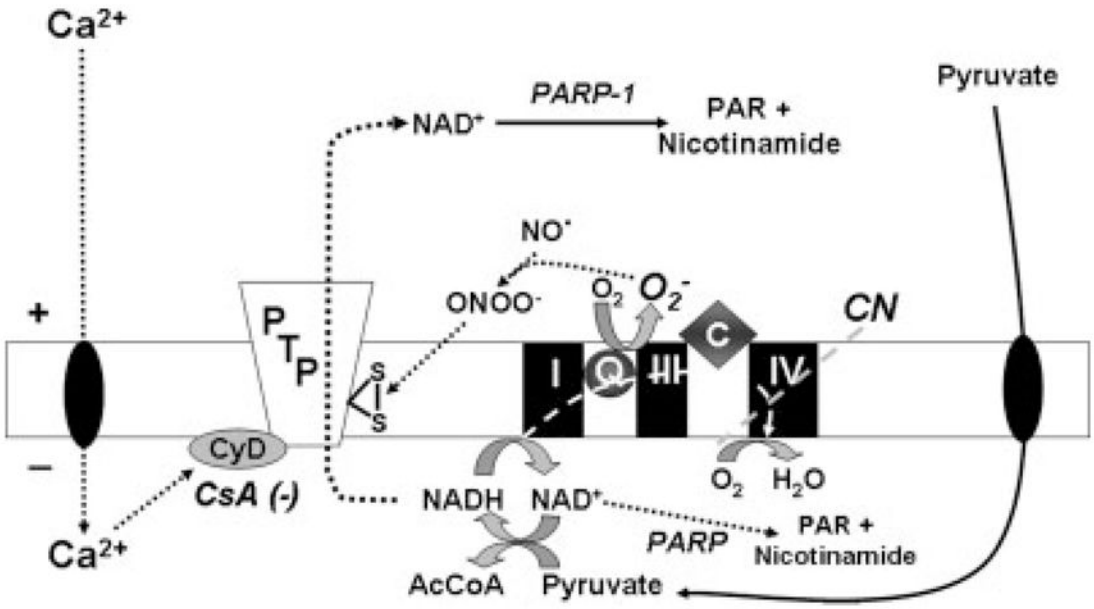

Fig. 3.

Oxidative stress–initiated metabolism of mitochondrial NAD by PARP-1. Inhibition of mitochondrial respiration by cyanide (CN) stimulates production of superoxide (O_2^-), which reacts with nitric oxide (NO·), forming peroxynitrite ($ONOO^-$), In the absence of reducing power to maintain sulfhydryl groups in a reduced redox state, peroxynitrite leads to net sulfhydryl oxidation. This oxidized redox state together with elevated intramitochondrial Ca^{2+} results in activation of the cyclophilin D (CyD)–dependent and cyclosporin A (CsA)-inhibitable permeability transition pore (PTP). Release of mitochondrial pyridine nucleotides through the PTP promotes further NAD metabolism by cytosolic/nuclear PARP-1. Activation of mitochondrial PARP may also contribute to NAD degradation. The presence of either exogenous or endogenous pyruvate helps to maintain a reduced mitochondrial redox state, thereby conferring protection against both PTP and PARP-1 activation.

TABLE I

Potential Factors Limiting Mitochondrial Energy Metabolism after Brain Injury

Factor	Sample references
Electron transport chain complexes	Allen et al. (1995) DiMauro and Schon (2003) Lin and Beal (2006)
Nitric oxide inhibition of cytochrome oxidase	Almeida et al. (2005) Jacobson et al. (2005)
Cytochrome c	Luetjens et al. (2000) Kirkinezos et al. (2005)
Mitochondrial matrix dehydrogenases	Tretter and Adam-Vizi (2000) Richards et al. (2006)
Adenine nucleotide translocase	Kurup et al. (1990) Vesce et al. (2005)
Mitochondrial porin	Vander Heiden et al. (2001) Colombini (2004)
NAD availability	Ying (2006) Cai et al. (2006)
Mitochondrial permeability transition	Kristian (2004) Sullivan et al. (2005)
Mitochondrial fission/trafficking	Yuan et al. (2006) Chang and Reynolds (2006)