Review

Neuroprotection through Stimulation of Mitochondrial Antioxidant Protein Expression

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Abstract. Oxidative stress and loss of cellular Ca^{2+} homeostasis are closely linked and are common denominators in the pathophysiology of many neurodegenerative diseases and acute disorders of the nervous system. Mitochondria are major targets of oxidative stress and abnormal intracellular Ca^{2+}, as both can cause bioenergetic failure through synergistic activation of the mitochondrial inner membrane permeability transition pore. Opening of this molecularly ill-defined pore causes both collapse of the membrane potential, which drives oxidative phosphorylation, and release of small metabolites, including pyridine nucleotides and glutathione, which are necessary for energy metabolism and defense against oxidative stress. Expression of genes coding for many antioxidant defense proteins is regulated by the Nrf2 transcriptional activating factor. Translocation of this protein from the cytosol to the nucleus is stimulated by oxidative stress and by specific agents that either react with cysteine sulfhydryl groups present on the protein KEAP1, that normally binds and restricts Nrf2 translocation, or that stimulate serine phosphorylation of Nrf2. Recent evidence indicates that mitochondria are a target of the cytoprotective gene expression induced by Nrf2 and that this pathway can increase resistance to redox-regulated opening of the permeability transition pore. Pharmacologic stimulation of the Nrf2 system and its protection against mitochondrial bioenergetic dysfunction may therefore constitute a powerful mechanism for both pre-conditioning against neurodegeneration and for post-conditioning against neural cell death associated with acute neurologic injury.

Keywords: Antioxidant, bioenergetics, calcium, cerebral ischemia, neurodegeneration, Nrf2, oxidative stress, permeability transition pore

OXIDATIVE STRESS AND MITOCHONDRIAL DYSFUNCTION IN NEURODEGENERATION

Figure 1 summarizes many of the known targets of reactive oxygen and nitrogen species (ROS/RNS) that are located at mitochondria or that are extramitochondrial but have strong secondary effects on mitochondrial bioenergetic or apoptotic activities. The most acute influence of ROS/RNS on mitochondria is mediated by oxidative modifications of proteins present in the electron transport chain (ETC) [1,2], other metabolic proteins, e.g., pyruvate dehydrogenase aconitase and α-ketogluatarate dehydrogenase [3–5], and the inner membrane permeability transition pore (PTP) [6,7]. Oxidation of cardiolipin, a phospholipid primarily lo-

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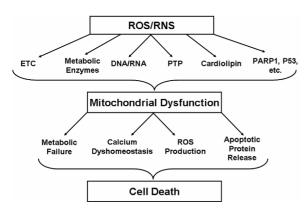
Fig. 1. Mitochondrial targets of reactive oxygen and nitrogen species and their effects on mitochondrial bioenergetic and apoptotic activities.

cated in the mitochondrial inner membrane, can also cause rapid mitochondrial functional alterations, including stimulated release of mitochondrial apoptotic proteins [8,9]. Oxidation of mitochondrial mRNA can limit the expression of any one of the 13 polypeptides coded for by the mitochondrial genome, resulting in delayed bioenergetic impairment [10]. Metabolic depression and increased ROS production by the ETC also occurs in response to mitochondrial DNA oxidation, which is associated with neurodegenerative disorders and normal aging [11]. Finally, ROS/RNS are known to stimulate the activity of poly-ADP ribose polymerase 1 (PARP1) and the expression of P53, which in turn can cause release of mitochondrial apoptotic proteins and poly-ADP ribosylation of mitochondrial proteins [12–15].

Several lines of evidence support the hypothesis that oxidative stress and associated mitochondrial bioenergetic dysfunction and activation of apoptosis are common etiological factors in many neurodegenerative diseases and acute disorders of the central nervous system (CNS) [16]. Descriptive experimental support includes the findings that mitochondrial morphology is altered, that metabolic activities are depressed, and that mitochondrial pro-apoptotic proteins are released to the cytosol prior to the death of neurons and other brain cells in both animal and cell culture models of neurodegeneration [17–22]. Biochemical markers of oxidative stress often exhibit close tempero-spatial relationships with these indicators of mitochondrial dysfunction [10, 23–25]. Moreover, agents or conditions that either decrease the production of reactive oxygen or nitrogen species (ROS/RNS) or increase their detoxification both ameliorate the mitochondrial functional anomalies and provide protection against subsequent cell death

and neurologic impairment. Such agents include novel antioxidants that are both lipophilic and that have a net positive charge, enabling them to be selectively accumulated within energized mitochondria, which possess a negative inside membrane potential [26,27].

One fundamental environmental factor that influences mitochondrial oxidative stress is oxygen. For example, hyperoxic reperfusion immediately after global cerebral ischemia increases oxidative protein and lipid oxidation, impairs mitochondrial respiration and cerebral energy metabolism, exacerbates delayed neuronal death, and worsens neurologic outcome [28–30]. In contrast to interventions utilizing exogenous antioxidants, avoiding unnecessary hyperoxia under conditions where cells are particularly vulnerable to oxidative stress likely improves outcome by reducing the production of ROS/RNS, due to simply restricting the concentration of O_2 available for reactions that produce superoxide and nitric oxide [31]. Remarkably, hypoxia can also promote mitochondrial oxidative stress when the concentration of O_2 is below the level necessary for sustaining normal respiration. Hypoxic oxidative stress is promoted by nitric oxide, which competes with O_2 at cytochrome oxidase, the terminal reaction of the ETC. This form of respiratory inhibition causes a reduced shift in the oxidation/reduction state of ETC redox centers capable of reducing O_2 to superoxide, increasing the production of this free radical and its metabolites, resulting in oxidative stress, even at very low O_2 levels [32].

In addition to the correlative evidence provided by comparisons between markers of oxidative stress, mitochondrial dysfunction, and cell death or neurologic outcome, genetic manipulation of proteins involved in both the production and detoxification of ROS/RNS and important mitochondrial targets of oxidative stress has provided independent evidence for their pathophysiological importance. For instance, overexpression of certain mitochondrial uncoupling proteins appears to both reduce production of ROS and provide neuroprotection [33–36]; however, a direct link between respiratory uncoupling and these two activities has not been proven conclusively [37]. Stronger molecular mechanistic evidence comes from knockouts and overexpression of the mitochondria-specific manganese superoxide dismutase (MnSOD). Genetically modified mice that overexpress or are deficient in MnSOD display resistance or vulnerability, respectively, to both oxidative stress and neurodegeneration in models of Alzheimer's and Parkinson's diseases and stroke [38–41]. An example of a critically important mitochondrial target of ox-

idative stress is the inner membrane permeability transition pore [42], as discussed in more detail below. Genetically modified mice that do not express cyclophilin D (CyD), a protein that promotes activation of the PTP, exhibit attenuated brain injury in models of stroke and multiple sclerosis [43,44]. Moreover, cells from CyD knockout mice are much more resistant to death induced by hydrogen peroxide than those from wild-type animals, indicating a role for CyD and the PTP in cell death induced by oxidative stress [43].

Examples of how experimental manipulation of genes that code for antioxidant enzymes or mitochondrial targets of oxidative stress can influence neurodegeneration are surpassed by the number of antioxidant and other cytoprotective genes that are both induced by endogenous oxidative stress and by exposure of cells or animals to certain drugs and environmental conditions. A master regulator of this system is the transcriptional activating factor, Nrf2.

THE Nrf2/ARE SYSTEM

Nrf2/ARE regulation

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a nuclear transcription factor that belongs to the "cap-'n collar" family that share a conserved basic leucine zipper (bZip) structure [45]. Under normal conditions, Nrf2 is kept inactive by being bound to Kelch like ECH-associated protein (KEAP1). KEAP1 is localized in the cytosol where it is bound to the actin cytoskeleton and targets Nrf2 for proteosomal degradation by being a Cul3-based E3 ligase adaptor. In the presence of ROS/RNS or certain electrophilic organic compounds, specific cysteine residues on KEAP1 are oxidized, causing a conformational change in KEAP1 and the release of Nrf2 into the cytosol. Oxidative stress also activates specific protein kinases, e.g., protein kinase c (PKC), which serine phosphorylate Nrf2, facilitating dissociation from KEAP1 and enabling transport of Nrf2 into the nucleus [45]. Electrophiles can also oxidize critical cysteine sulfhydryl groups present on Nrf2, masking the nuclear export signal sequence and allowing Nrf2 to remain within the nucleus long enough to activate gene transcription [46]. Within the nucleus, Nrf2 forms heterodimers with sMAF proteins that also stabilize nuclear retention. These heterodimers bind to antioxidant response element (ARE) sequences and recruit transcriptional enzymes and other proteins to these locations [47]. Since an ARE sequence is locat-

ed proximal to the Nrf2 gene, Nrf2 activation acts in a positive, feed-forward manner [48]. To prevent continuous transcriptional activation of this and many other genes, nuclear tyrosine kinases phosphorylate Nrf2, stimulating its translocation out of the nucleus back to the cytosol [49].

Transcriptional regulation by Nrf2

While Nrf2 is widely known for its regulation of phase II detoxification enzymes, it has transcriptional control over many genes that include an ARE sequence in their promoter region. Some of the commonly known genes it regulates include NAD(P)H:quinone oxidoreductase 1 (NQO1), heme oxygenase 1 (HO-1), glutathione S-transferases (GST), glutathione synthetic enzymes, antioxidant enzymes, and NADPH regenerating enzymes, e.g., glucose-6-phosphate dehydrogenase and malic enzyme [50]. In various proteomic studies, Nrf2 has been shown to control genes involved in immunity, membrane transport, cell adhesion, cell cycle, and energy metabolism, among others [51]. Numerous kinases and phosphatases are also under the regulation of Nrf2, including mitogen activated protein kinase (MAPK), serine kinases, and tyrosine phosphatases [51]. Several Nrf2-regulated genes code for important regulators of cellular energy metabolism, including the mitochondrial enzymes pyruvate dehydrogenase lipoamide β and pyruvate dehydrogenase kinase. Nrf2 also exerts control over aquaporin, multiple classes of ATPases, chloride, potassium, and calcium and folate channels [50,51].

INDUCTION OF THE Nrf2/ARE PATHWAY

Pharmacologic activation of the Nrf2/ARE pathway

This field of research was spawned by the observation that when mice were fed chow containing both carcinogens plus the electrophilic preservative butylated hydroxyanisole (BHA), they were protected from stomach cancer that was induced by the carcinogens in the absence of BHA [52]. Subsequently, it was determined that this inducible xenobiotic response was mediated by phase II detoxification enzymes and that the Nrf2/ARE pathway was responsible for inducing the expression of genes coding for these proteins [48]. This discovery led to the search for and identification of compounds that could safely activate the Nrf2/ARE pathway of gene expression [53].
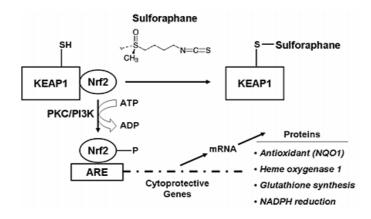
Fig. 2. Activation of Nrf2/Antioxidant Response Element (ARE) pathway of gene expression. Two primary mechanisms of activation include oxidation of specific cysteine sulfhydryl groups on KEAP1, e.g., by reaction with sulforaphane, and serine phosphorylation of Nrf2 by enzymes including protein kinase c (PKC) and phosphatidlyinositol 3-kinase (PI3K).

Electrophilic activation

Sulforaphane is a compound derived from a glucosinolate found in cruciferous vegetables such as broccoli and kale in the *Brassica* genus. When these foods are consumed, the salivary enzyme myrosinase converts the inactive glucosinolate, glucoraphanin, into its active isothiocyanate form, sulforaphane (see Fig. 2). The central carbon of its electrophilic cyanate group, –N=C=S, reacts with nitrogen, sulfur, and oxygen based nucleophilies [54]. Following rapid uptake of sulforaphane into tissues and cells, this cyanate group reacts rapidly with three critical cysteines, C^{151} C^{273} C^{288}, on KEAP1, causing a conformational change that reduces its affinity for Nrf2. Nrf2 is released into the cytoplasm, enters the nucleus, and activates ARE-driven genes [55]. While traditional focus has been on KEAP1 as the redox sensor mediating Nrf2 translocation, Nrf2 itself may be the key redox sensor and thus control its own nuclear translocation. Central to this model is a single cysteine, C^{183}, present within the nuclear export signal sequence located in the transactivation domain (NES_{TA}) of Nrf2. Experiments using expression of a GFAP-tagged, truncated Nrf2 containing the NES_{TA} demonstrate dose-dependent translocation of the protein by SFP. When this cysteine is mutated to an alanine, the protein is unable to translocate to the nucleus, thus identifying C^{183} as a critical redox sensor on Nrf2. In summary, SFP oxidatively modifies KEAP1 and reduces its affinity for Nrf2. Nrf2 released into the cytosol is then oxidized by sulforaphane, promoting its entry into the nucleus [46]. Other electrophilic compounds that appear to act by the same mechanism as that of sulforaphane include carnosic acid [56] and curcumin [57].

Activation of Nrf2 by phosphorylation events

In addition to redox modulation of KEAP1 and Nrf2, phosphorylation of Nrf2 facilitates its stabilization and translocation into the nucleus [45]. Treatment of various cell lines with either tert-butyl hydroquinone (tB-HQ) [58] or plumbagin [59], a pigment extracted from the Plumbaginaceae family of flowering plant that contains anti-microbial properties, results in activation of the PI3K/AKT signaling cascade, causing phosphorylation and activation of Nrf2 [59]. If PI3K inhibitors wortmannin or LY294002 are present, Nrf2 translocation is abrogated [59,60]. PKC mediates phosphorylation of Nrf2 at serine 40 [61]. While phosphorylation of serine 40 is not required for translocation of Nrf2 into the nucleus, it plays a critical yet still undefined role in the interaction between KEAP1 and Nrf2 [61]. One model states that phosphorylation of Nrf2 is absolutely required for release of Nrf2 from KEAP1 [61]. An alternative model contends that phosphorylated free Nrf2 is unable to bind KEAP1 therefore stabilizing Nrf2 and contributing to the increase of free Nrf2 in the cytoplasm [48,62]. Beyond cytoplasmic stabilization of Nrf2, PKC contributes to Nrf2 nuclear retention by phosphorylating GSK3β thus inhibiting the GSK3β/Fyn signaling cascade, which is responsible for nuclear export of Nrf2 [62].

ROLE OF Nrf2 IN NEUROPROTECTION

Acute CNS injury

Several different animal models of acute brain injury have demonstrated neuroprotection by pharmacologic

activation of the Nrf2/ARE pathway of gene expression. In a rat model of intracerebral hemorrhage (ICH) using extravasated blood, post-hemorrhagic administration of sulforaphane is followed by translocation of Nrf2 to the nucleus, an increase in mRNA levels for superoxide dismutase 1 (SOD1), GST and NQO1, and an improvement in neurologic outcome [63]. Using the same ICH model in mice, post-treatment with sulforaphane resulted in improvements in wild-type animal's neurological deficit score while Nrf2 knockout mice had a worse score and sulforphane no longer had any neuroprotective affects [63]. In a separate ICH model where collagenase is used, Nrf2 knockout mice exhibit increased brain injury, worse neurological outcome, and increased evidence of oxidative stress, compared to Nrf2 wild-type mice [64]. In a rat focal ischemic stroke model, sulforaphane post-treatment reduces brain infarct volume and increases the levels of both HO-1 mRNA and protein [65]. Nrf2 knockout mice display an increase in infarct volume and worsened neurologic outcome, compared to wild-type mice [66,67]. Neuroprotection following ischemic stroke has also been demonstrated by administration of carnosic acid, which also activates the Nrf2 pathway [56].

Intraperitoneal administration of sulforaphane to rats or mice after traumatic brain injury results in an increase in mRNAs for various phase II response enzymes and reduced impairment of memory and cognition [68–70]. Importantly, Nrf2 knockout mice do not demonstrate improved outcome when treated with sulforaphane [70]. Preliminary results also indicate neuroprotection by sulforaphane post-treatment in a large animal model of acute brain injury. Specifically, intravenous administration of sulforaphane at 1 mg/kg to adult beagles at 30 min after cardiac arrest and resuscitation reduces hippocampal neuronal death and improves neurologic outcome [71]. *In vitro* experiments suggest that the neuroprotective effects of sulforaphane may target both neurons and glia. Pre- or post-treatment of cortical astrocytes or hippocampal neurons with sulforaphane increases Nrf2-regulated expression of NQO1, HO-1, among other genes, and reduced delayed cell death following transient O_2 and glucose deprivation [72,73]. In an organotypic culture model of spinal cord injury, results indicate that pharmacologic activation of the Nrf2 pathway prevents motor neuron cell death, upregulates Nrf2 and HO-1 mRNA levels, reduces toxic extracellular levels of glutamate, and preserves mitochondrial ultrastructure [74].

The success of this approach in various *in vivo* and *in vitro* models is likely due to the multiple antioxidant and other cytoprotective genes that are induced. This strategy thus represents a novel "combination therapy" approach and may therefore be more effective or broadly applicable than the use of antioxidant drugs that normally target only one or a few specific ROS or RNS. Taken together, these findings demonstrating neuroprotection by sulforaphane post-treatment as well as pre-treatment in different models of acute CNS injury provide optimism that pharmacologic activation of the Nrf2/ARE system will eventually be translated to effective clinical therapeutic interventions.

Neurodegenerative diseases

Neuroprotection through upregulation of the Nrf2 pathway of cytoprotective gene expression is also applicable to neurodegenerative diseases. Alzheimer's disease (AD) is characterized histologically by amyloid-β (Aβ) plaques and neurofibrillary tangles in various brain locations. In an *in vitro* model of AD cytopathology, treatment of differentiated NT2N neurons with 4-hydroxynonenal induces formation of Aβ tangles. When these cultures are pre-treated with the pro-oxdiant, tert-butyl hydroquinone, Nrf2 is activated, followed by decreased Aβ formation and caspase 3 activation [75]. In a transgenic mouse AD model expressing mutated human amyloid-β protein precursor and presenilin 1 genes, intra-hippocampal injections of lentiviruses containing the Nrf2 gene result in elevated HO-1 gene expression and improved spatial learning memory [76]. In a mouse cytotoxin (malonate) model of Huntington's disease, neuroprotection is observed by intrastriatal implantation of astrocytes with activated Nrf2-mediated gene expression [77]. Pretreatment of either dopaminergic cell lines or nigrostriatal co-cultures with sulforaphane provides neuroprotection against Parkinson's disease neurotoxins, e.g., 6-hydroxydopamine [78,79]. Mechanisms of neuroprotection in these models may include the increased expression of NQO1, which prevents ROS production that is catalyzed by the semiquinone fraction of the increased pool of quinones generated by aberrant dopamine metabolism, and increased levels of glutathione GSH, which detoxify peroxides and protect against oxidation of protein sulfhydryl groups [79]. In a MPTP mouse model of Parkinson's disease, overexpression of Nrf2 in astrocytes prevents neuronal death and reduces inflammation [80]. In a mouse model of amyotrophic lateral sclerosis, overexpression of Nrf2 in astrocytes improves neuronal survival and slows disease progression [81]. Additional evidence for the im-

portance of Nrf2 in maintaining resistance to neurodegeneration comes from models where outcome is worsened by knockout of Nrf2 gene expression, e.g., in the malonate model of Huntington's disease [77], the MPTP model of Parkinson's disease [80,82], and in a kainate-induced status epilepticus mouse model [83]. The experimental evidence therefore strongly supports an important role of Nrf2 and its regulation of cytoprotective gene expression in both resistance to neurological disorders and in response to exogenous agents that can protect against or slow the progression of neurodegenerative diseases.

Nrf2-REGULATED ANTIOXIDANT DEFENSE SYSTEMS

All tissues possess redundant antioxidant defense systems for detoxifying the ROS and RNS that are constantly present and that play important roles as intracellular and transcellular regulatory signals. Considerable effort has been made to characterize these systems in the brain, knowing that oxidative stress plays a major role in most forms of both acute CNS injury and in chronic neurodegenerative disorders. It was recognized early during these investigations that the gene expression for many of the antioxidant associated proteins is upregulated in response to oxidative and other forms of stress. For instance, HO-1 and SOD2 gene induction occurs rapidly following focal cerebral ischemia [84,85]. These observations taken together with those demonstrating stress-induced increased expression of anti-apoptotic genes, e.g., Bcl-2, led to the concept of genomic preconditioning against brain injury, using levels of stress that are sufficient to activate cytoprotective gene expression but that are also below the threshold for inducing significant cell dysfunction or death.

It now appears that at least some if not many of the genes that are induced in the brain following both toxic and sub-toxic levels of stress may be activated via the Nrf2/ARE system [61]. Products of these genes include SOD1 (cytosolic), SOD2 (mitochondrial), and SOD3 (extracellular) [86,87] and the peroxide detoxifying enzymes glutathione peroxidase [88] and catalase [89,90]. Nrf2 also mediates induction of glutamate-cysteine ligase catalytic subunit (GCLC) and glutamate-cysteine ligase, modifier subunit (GCLM), both of which are necessary for glutathione biosynthesis [91]. NAD(P)H is critical to antioxidant detoxification as it provides the reducing power needed to keep glutathione reduced.

Malic enzyme (ME) and hexose monophosphate shunt dehydrogenases are the primary producers of cytosolic NADPH and are induced by Nrf2 in the small intestine but their induction has yet to be reported for brain [50]. Another gene subject to Nrf2 control is HO-1, which catalyses the catabolism of heme to carbon monoxide (CO), biliverdin and iron [92]. Upregulation of HO-1 [65] leads to increased bilirubin levels, a product of oxidized biliverdin, which is a potent antioxidant. The CO created by HO-1 indirectly activates the AKT signaling pathway that potentiates Nrf2 cytoprotection by at least two mechanisms: First, it prevents the phosphorylation of GSK-3β, promoting nuclear retention of Nrf2 and therefore transcription of Nrf2 driven genes [92]. Secondly, it promotes the translocation of nuclear respiratory factor 1 (Nrf1) to the nucleus which in turn stimulates the transcription and translation of Tfam, that stimulates transcription replication of the mitochondrial genome, promoting mitochondrial biogenesis [92]. Finally, NQO1, which is perhaps the most commonly used marker of Nrf2 activated gene expression, directly reduces semiquinones to hydroquinones, which inhibits superoxide production catalyzed by semiquinone radicals and provides the fully reduced quinones that function as free radical scavengers [93].

MITOCHONDRIAL ANTIOXIDANT DEFENSES AND POTENTIAL REGULATION BY Nrf2

The majority of ROS that are produced the in the mitochondria are a byproduct of the respiratory chain and specific dehydrogenases, e.g., α-ketoglutarate dehydrogenase [94–99]. The primary species generated is the superoxide radical O_2^-. As shown in Fig. 3, under normal conditions, the O_2^- is converted to H_2O_2 by SOD2. Another important source of mitochondrial H_2O_2 is monoamine oxidase [100,101]. H_2O_2 is further converted to H_2O, by catalase, and glutathione or thioredoxin peroxidases at the expense of either reduced glutathione (GSH) or reduced thioredoxin (TrxSH). GSSG or TrxS$^-$ are reduced by their respective glutathione or thioredoxin reductases, using NADPH as the electron donor [102]. The NADPH pool in the mitochondria is maintained mostly through the activities of several enzymes, including transhydrogenase, mitochondrial malic enzyme (ME3), and NADP$^+$-selective isocitrate and glutamate dehydrogenases.
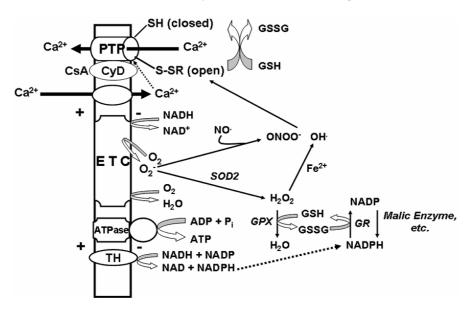
Fig. 3. Redox regulation of the mitochondrial permeability transition pore (PTP). Opening of the PTP is promoted by elevated intramitochondrial Ca^{2+} and by conditions that promote protein sulfhydryl oxidation, including the presence of peroxynitrite ($ONOO^-$) and hydroxyl radical ($OH^.$) and a relatively low ratio of reduced to oxidized glutathione (GSH/GSSG). The glutathione redox state is determined by the balance between its reduction by the NADPH-dependent glutathione reductase (GR) and its oxidation, e.g., by glutathione peroxidase (GPX). Intramitochondrial $NADP^+$ is reduced to form NADPH by several enzymes including the inner membrane potential-driven transhydrogenase (TH) and cell-type selective isoenzymes, including malic enzyme 3 and $NADP^+$-dependent isocitrate dehydrogenase glutamate dehydrogenase. The presence of elevated peroxides, including hydrogen peroxide (H_2O_2) generated e.g., by mitochondrial superoxide dismutase (SOD2) promote PTP opening by shifting the mitochondrial redox state, including that of glutathione, to a more oxidized level. PTP opening is subject to pharmacological inhibition, e.g., by the interaction of cyclosporin A with cyclophilin D, a protein that regulates but is not equivalent to the PTP.

Oxidative stress occurs within the mitochondria, as it does in other cellular locations, when the rate of ROS production and ROS detoxification are unbalanced. Increased production of toxic superoxide and consequently hydrogen peroxide can exceed their rate of detoxification, resulting in toxic metabolites, e.g., peroxynitrite and hydroxyl radical, that can cause mitochondrial dysfunction through the oxidation of various proteins, lipids and nucleic acids as described earlier (see Fig. 1). However, growing evidence suggests that protection against ROS accumulation can occur through increased expression of mitochondrial antioxidant enzymes, e.g., SOD2, or proteins responsible for the biosynthesis of glutathione and for the reduction of $NADP^+$. Levels of SOD2 and mitochondrial glutathione peroxidase and reductase and glutathione are elevated by treatment of aortic smooth muscle cells and cardiac myocytes with sulforaphane [103] or dithiolethione, another organosulfur compound [104]. While these effects have not yet been reported for mitochondria present within neurons or the CNS, preliminary results from our lab support this hypothesis and demonstrate that mitochondria from the brains of rats treated with sulforaphane are resistant to t-butyl hydroperoxide-induced opening of the PTP [71]. Possible explanations for this indirect effect of sulforaphane include increased expression of mitochondrial enzymes that reduce $NADP^+$ to NADPH [105] and increased expression of the anti-apoptotic mitochondrial protein, Bcl-2. Cardiac Bcl-2 levels are elevated in rats maintained on a high broccoli diet [106]. Moreover, we have shown that mitochondria within Bcl-2 overexpressing neural cell lines are resistant to PTP opening [107]. Irrespective of the mechanism by which sulforaphane inhibits PTP opening, this effect could contribute to the cytoprotective effects of this drug and others that act through the Nrf2/ARE system, since PTP opening contributes to bioenergetic failure and cell death in many models of acute and chronic neurodegeneration and in cell death that occurs in the cardiovascular, renal, and other systems [108–110].

SUMMARY

Mitochondria are both important producers of ROS/RNS and very sensitive to damage caused by these molecules either directly or through the oxidative stress

associated with their metabolism. Exogenous antioxidants, including those that are targeted to mitochondria, can increase mitochondrial resistance to oxidative damage and consequently provide neuroprotection for neurologic disorders and diseases. Another approach, which is also showing great promise, is pharmacologic activation of endogenous antioxidant gene expression, including that which is controlled by the Nrf2/ARE system. Preliminary evidence indicates that this strategy can protect mitochondria against oxidative damage and bioenergetic dysfunction; however, the molecular mechanisms responsible for this defense have yet to be elucidated.

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