

Mitochondrial Mechanisms of Neural Cell Death and Neuroprotective Interventions in Parkinson's Disease

GARY FISKUM,^a ANATOLY STARKOV,^{a,b} BRIAN M. POLSTER,^{a,c}
AND CHRISTOS CHINOPOULOS^a

^a*Department of Anesthesiology, University of Maryland School of Medicine,
Baltimore, Maryland 21201, USA*

^c*Department of Molecular Microbiology and Immunology,
The Johns Hopkins University School of Public Health,
SHPH 5132, 615 N. Wolfe Street, Baltimore, Maryland 21205-2179, USA*

ABSTRACT: Mitochondrial dysfunction, due to either environmental or genetic factors, can result in excessive production of reactive oxygen species, triggering the apoptotic death of dopaminergic cells in Parkinson's disease. Mitochondrial free radical production is promoted by the inhibition of electron transport at any point distal to the sites of superoxide production. Neurotoxins that induce parkinsonian neuropathology, such as MPP⁺ and rotenone, stimulate superoxide production at complex I of the electron transport chain and also stimulate free radical production at proximal redox sites including mitochondrial matrix dehydrogenases. The oxidative stress caused by elevated mitochondrial production of reactive oxygen species promotes the expression and (or) intracellular distribution of the proapoptotic protein Bax to the mitochondrial outer membrane. Interactions between Bax and BH3 death domain proteins such as tBid result in Bax membrane integration, oligomerization, and permeabilization of the outer membrane to intermembrane proteins such as cytochrome c. Once released into the cytosol, cytochrome c together with other proteins activates the caspase cascade of protease activities that mediate the biochemical and morphological alterations characteristic of apoptosis. In addition, loss of mitochondrial cytochrome c stimulates mitochondrial free radical production, further promoting cell death pathways. Excessive mitochondrial Ca²⁺ accumulation can also release cytochrome c and promote superoxide production through a mechanism distinctly different from that of Bax. Ca²⁺ activates a mitochondrial inner membrane permeability transition causing osmotic swelling, rupture of the outer membrane, and complete loss of mitochondrial structural and functional integrity. While amphiphilic cations, such as dibucaine and propranolol, inhibit Bax-mediated cytochrome c release, transient receptor potential channel inhibitors inhibit mitochondrial swelling and cytochrome c release induced by the inner membrane permeability transi-

Address for correspondence: Dr. Gary Fiskum, Dept. of Anesthesiology, Univ. of Maryland School of Medicine, 685 W. Baltimore St., MSTF 5.34, Baltimore, MD 21201. Voice: 410-706-3418; fax: 410-706-2550;

Gfisk001@umaryland.edu

^bCurrent address: Department of Neurology, Weil Medical College, Cornell University, 510 E. 70th St., New York, NY 10021.

Ann. N.Y. Acad. Sci. 991: 111–119 (2003). © 2003 New York Academy of Sciences.

tion. These advances in the knowledge of mitochondrial cell death mechanisms and their inhibitors may lead to neuroprotective interventions applicable to Parkinson's disease.

KEYWORDS: apoptosis; cytochrome c; calcium; excitotoxicity; Bax

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disease characterized clinically by bradykinesia, rigidity, resting tremor, and ataxia. These symptoms are caused by decreased dopamine release in the striatum. Pathologically, PD is characterized primarily by the death of dopaminergic neurons in the substantia nigra pars compacta and the formation of ubiquitin- and α -synuclein-positive cytoplasmic inclusions (Lewy bodies). The molecular mechanisms responsible for these changes are not clearly understood. One theory is that mitochondrial dysfunction, due to either environmental or genetic factors, results in excessive oxidative stress that triggers apoptotic cell death.

EVIDENCE FOR A MITOCHONDRIAL ETIOLOGY OF PARKINSON'S DISEASE

Several lines of evidence support the hypothesis that mitochondrial dysfunction contributes to the etiology of Parkinson's disease. Electron transport chain complex I activity is reduced in PD substantia nigra autopsy specimens as well as in PD platelets.^{1,2} A mitochondrial genomic etiology for defective complex I in PD is strongly suggested by the presence of altered complex I activity, abnormal mitochondrial morphology, and impaired mitochondrial energy-dependent activities in cybrid cell lines containing a normal nuclear genome but mitochondrial DNA from PD patients.³⁻⁵ Parkinson's disease cybrids are also more sensitive to death induced by MPP⁺, a dopaminergic neuron-selective toxin that induces Parkinson-like lesions and symptoms in both humans and animals.⁴ Additional evidence for a mitochondrial etiology of PD is the finding that chronic systemic treatment of rats with rotenone, a highly specific complex I inhibitor, can induce Lewy body neuropathology in addition to nigrostriatal dopaminergic degeneration and neurologic features of PD.⁶

MITOCHONDRIAL INITIATION OF NECROTIC AND APOPTOTIC CELL DEATH

Mitochondria have long been considered as mediators of cell death in neurodegenerative disorders. The significance of mitochondrial injury was previously thought to be limited to the potential effects such injury has on maintaining sufficient cellular ATP to avoid necrotic cell death. However, we now understand that mitochondria are the primary mediators of cell death caused by abnormal levels of intracellular Ca²⁺ elicited during excitotoxicity⁷ and that mitochondrial mechanisms of neural cell death include oxidative stress and apoptosis in addition to metabolic failure (FIG. 1). Relatively mild mitochondrial injury, where ATP levels are maintained

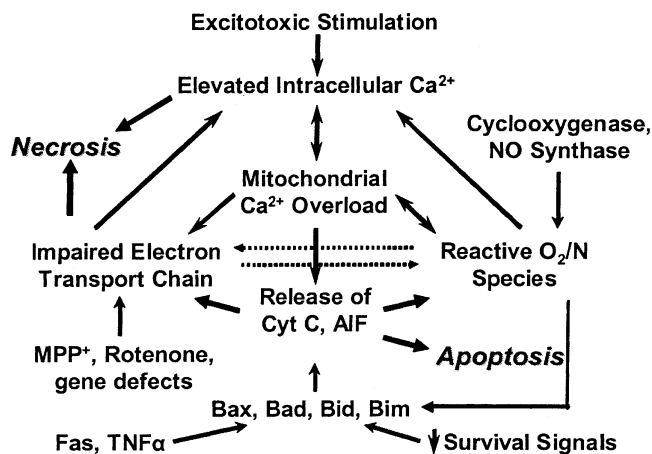


FIGURE 1. Mitochondrial mechanisms of neural cell death. Excitotoxic levels of intracellular Ca^{2+} accumulate within mitochondria, potentially causing metabolic failure, oxidative stress, and apoptosis via the release of cytochrome c and other proapoptotic mitochondrial proteins. Proteins, such as Bax, also mediate the release of mitochondrial cytochrome c, which, in addition to activating the caspase apoptotic cascade, can also stimulate mitochondrial free radical production. The levels and (or) subcellular distribution of these proteins is under the control of trophic factors and is also affected by oxidative stress. Mitochondrial free radical production is stimulated by the neurotoxins MPP^+ and rotenone and may be elevated by genomic or environmentally mediated alterations in electron transport chain activities.

near normal, results in mainly apoptotic cell death. More extensive injury that causes ATP depletion shifts the form of cell death toward necrosis. Excessive accumulation of Ca^{2+} that occurs during excitotoxic stimulation is likely not the only mediator of mitochondrial injury. Mitochondria are the targets of reactive oxygen species (ROS) generated by a number of different systems, including the mitochondrial electron transport chain (ETC), cyclooxygenases, Fe^{2+} -catalyzed hydroxyl radical (OH^{\bullet}) formation, and peroxynitrite formed from the reaction of nitric oxide (NO^{\bullet}) with superoxide ($\text{O}_2^{\bullet-}$). The levels and activities of mitochondrial antioxidant defense systems—for example, superoxide dismutase and glutathione peroxidase—and the redox state of mitochondrial NAD(P)H are therefore extremely important determinants of the extent of oxidative mitochondrial injury and neural cell survival.

THE INTRINSIC MITOCHONDRIAL PATHWAY OF APOPTOSIS

Discovery of the involvement of the release of mitochondrial cytochrome c in the activation of the cell death protease (caspase) cascade leading to apoptosis is one of the most important and certainly most unexpected events in the history of cell death research (FIG. 2). Release of several proapoptotic mitochondrial proteins, such as cytochrome c and apoptosis initiating factor (AIF), and their redistribution to the cytosol and nucleus during neural cell death *in vitro* and *in vivo* are well documented.⁷ Sev-

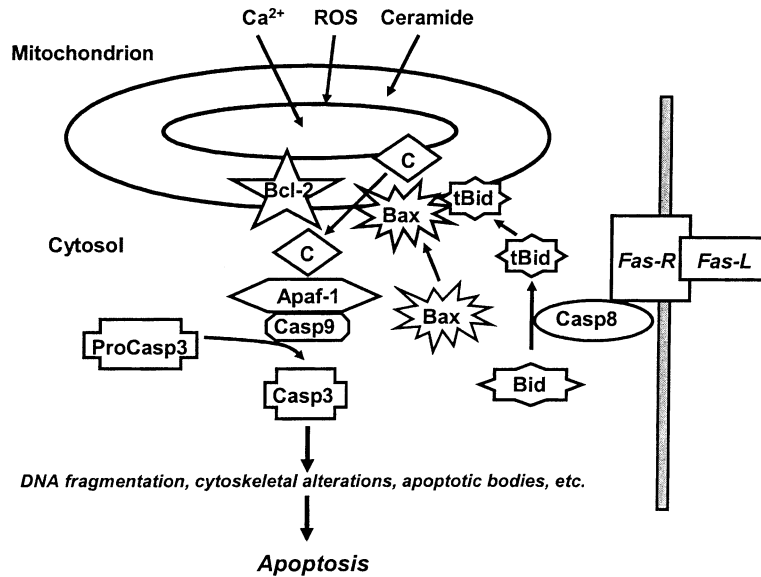


FIGURE 2. Mitochondrial participation in apoptosis. Agents, such as Ca^{2+} , ROS, and ceramide, as well as proapoptotic proteins, such as Bax, stimulate the release of other proapoptotic proteins—for example, cytochrome c (C in the figure)—from the mitochondrial intermembrane space into the cytosol. Cytochrome c and procaspase 9 together with apoptosis activating factor 1 (Apaf-1) form a multiprotein complex (apoptosome) that activates caspase 9, which then cleaves procaspase 3, forming active caspase 3. This caspase, together with other caspases it activates, proteolytically degrades a variety of proteins, causing the molecular and morphological alterations characteristic of apoptosis. Cell surface “death receptors,” such as Fas, can activate caspase 3 directly through activation of caspase 8 (not shown) or participate in the mitochondrial pathway by processing Bid to form tBid, which greatly stimulates the release of cytochrome c by Bax. The antiapoptotic protein Bcl-2 is capable of inhibiting the release of cytochrome c mediated by either Ca^{2+} or Bax.

eral factors are capable of triggering the release of proapoptotic proteins from mitochondria, including elevated Ca^{2+} , ROS, ceramide, and other cell death proteins, such as Bax and tBid. Some apoptotic proteins, such as tBid, are activated by proteolytic cleavage and redistribute to the mitochondria in response to signals—for example, activation of the Fas and tumor necrosis factor (TNF)- α “cell death receptors.” In addition to setting the mitochondrial pathway in motion, these receptors can trigger apoptosis via an “extrinsic pathway” by stimulating caspase 8-mediated activation of caspase 3 through proteolytic cleavage. We obtained evidence for Fas receptor activation in the penumbra surrounding brain infarcts caused by closed head injury in humans.⁸ The involvement of Fas and TNF α receptor activation in PD dopaminergic cell death is less clear. While the ligands for these receptors and other inflammatory cytokines are elevated in nigrostriatal dopaminergic regions and in the cerebrospinal fluid of PD patients,⁹ these changes may reflect nonneuronal, proinflammatory cell activation.¹⁰ Despite this controversy, abundant evidence obtained from patients and from animal models indicates that apoptosis plays a critical role in PD dopaminergic

cell death and that the mitochondrial pathway of apoptosis is integrally involved.¹¹ Therefore, the molecular mechanisms responsible for the release of proapoptotic proteins from mitochondria are potential targets for therapeutic intervention in PD.

There are two fundamentally different mechanisms of mitochondrial protein release under consideration. The first involves physical disruption of the mitochondrial outer membrane and simple diffusion of cytochrome c from its normal exclusive location in the space located between the outer and inner mitochondrial membranes. The second mechanism involves transport of cytochrome c through a pore located in the outer membrane.

A likely cause for the physical disruption of the outer membrane is the osmotic swelling of the compartment surrounded by the inner membrane (matrix) due to an increased permeability of the inner membrane to small osmotically active solutes. There is a large body of literature describing this swelling phenomenon known as the mitochondrial membrane permeability transition (MPT).¹² This activity is triggered by abnormal mitochondrial Ca^{2+} accumulation and is promoted by oxidative stress and reactive metabolites, such as peroxynitrite. The MPT is generally defined as a relatively nonspecific increase in inner membrane permeability that results in a substantial increase in matrix volume and a decrease in mitochondrial membrane potential that can be inhibited by the presence of the drug cyclosporin A (CsA) and by overexpression of the antiapoptotic gene Bcl-2,¹³ both of which protect against MPP⁺-induced dopaminergic cell death.^{14,15} Although CsA is very effective at inhibiting the MPT for non neural cell mitochondria, Ca^{2+} -induced cytochrome c release from brain mitochondria is relatively resistant to inhibition by CsA in the presence of physiologically realistic concentrations of the cytosolic components Mg^{2+} and ATP.¹⁶

We recently found that 2-aminoethoxydiphenyl borate, an inhibitor of transient receptor potential (TRP) channels, is much more effective than CsA at protecting against Ca^{2+} -induced cytochrome c release from brain mitochondria.¹⁷ This observation and others suggest that the MPT is associated with activation of Trp channels, a potential new class of targets for neuroprotection in PD and other brain disorders.

The MPT is an attractive mechanism of acute neural injury due to its activation by factors known to be associated with excitotoxicity and because of its sensitivity to inhibition by certain drugs and gene products known to be neuroprotective. However, increasing evidence indicates that the mechanism by which proapoptotic proteins such as Bax and tBid release cytochrome c and cause other forms of mitochondrial dysfunction is independent of the MPT and involves either pore formation or lipid alterations at the mitochondrial outer membrane.^{18–20} The potential importance of Bax in PD is illustrated by the observation that Bax-knockout mice are resistant to nigrostriatal cell death induced by MPP⁺.²¹

The Bax-mediated mechanism of cytochrome c release is not inhibited by CsA but is inhibited by specific amphiphilic cations, such as dibucaine and propranolol, known to affect membrane lipid–protein interactions.²² In addition to activation of proapoptotic proteins like Bid by cell death receptors, expression of the genes coding for these proteins and mitochondrial–protein interactions are promoted by high Ca^{2+} and ROS through their stimulation of complex signal transduction cascades.²³ Therefore, Ca^{2+} together with oxidative stress can promote cytochrome c release and apoptosis by both the MPT- and Bax-mediated molecular mechanisms that exhibit different pharmacologic sensitivities.

Mechanisms of mitochondrial proapoptotic protein release in addition to the MPT- and Bax-dependent pathways should also be considered. As AIF release appears downstream of cytochrome c release and caspase activation,²⁴ it is possible that proteolytic cleavage of AIF or an anchoring protein might be necessary. Also, as cytochrome c release stimulates mitochondrial generation of reactive oxygen species,²⁵ the oxidative modification of mitochondrial membrane lipids or proteins could be another event responsible for or promoting release of proapoptotic mitochondrial proteins.

A possible key to the development of mitochondrial neuroprotective interventions is the understanding of the mechanisms by which the antiapoptotic, mitochondrial protein Bcl-2 inhibits cytochrome c release mediated by both MPT-dependent and -independent pathways. Bcl-2 and its close relative Bcl-X_L exert some form of antioxidant activity that confers cytoprotection against ROS that may also inhibit activation of the MPT.^{13,26} In contrast, Bcl-2 inhibition of Bax appears to involve direct protein-protein interaction, impairment of Bax oligomerization, and consequently inhibition of pore formation.²⁷ Stimulation of Bcl-2 expression has been demonstrated in several ischemic preconditioning paradigms and may represent a primary mechanism of neuroprotection by estrogen.²⁸ Investigators have also utilized protein transduction domains to deliver exogenous Bcl-X_L to cells throughout the brain and have demonstrated neuroprotection when these fusion proteins were administered by intraperitoneal injection up to 1 hour following the occlusion of the middle cerebral artery of mice.²⁹ Delivery of neuroprotective proteins, such as Bcl-2 and glial cell line-derived neurotrophic factor (GDNF), as a therapeutic approach for neurodegenerative disorders like Parkinson's disease is therefore possible and may prove to be effective.

MITOCHONDRIAL MECHANISMS OF REACTIVE OXYGEN SPECIES GENERATION

As several lines of evidence suggest that elevated mitochondrial ROS production contributes to the etiology of Parkinson's disease and as oxidative stress is a potent activator of apoptosis, understanding the mechanisms of mitochondrial free radical production is critically important in elucidating the pathophysiology of Parkinson's and other neurodegenerative diseases. The two most commonly cited sites of mitochondrial ROS production are ubiquinone (during complex III reduction) and complex I, although others, such as complex II, may also contribute.³⁰ The site of mitochondrial ROS production implicated most strongly in Parkinson's disease is complex I of the electron transport chain. Some neurotoxins that induce PD neuropathology *in vivo*, such as MPP⁺ and rotenone, are inhibitors of complex I and stimulate ROS generation *in vitro*.³¹ Acting along with hydroxyl radical (OH[•]) and peroxynitrite (HNOO⁻), these ROS species can cause oxidative damage and inhibition of mitochondrial enzyme activities, including those of complex I and alpha-ketoglutarate dehydrogenase complex (α KGDC) (FIG. 3).^{32,33} This inhibition can lead to metabolic failure through impairment of electron transport-dependent generation of the proton-motive force that drives the synthesis of ATP. Complete detoxification of superoxide depends on the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPX), and glutathione reductase (GR) together with glutathione and a

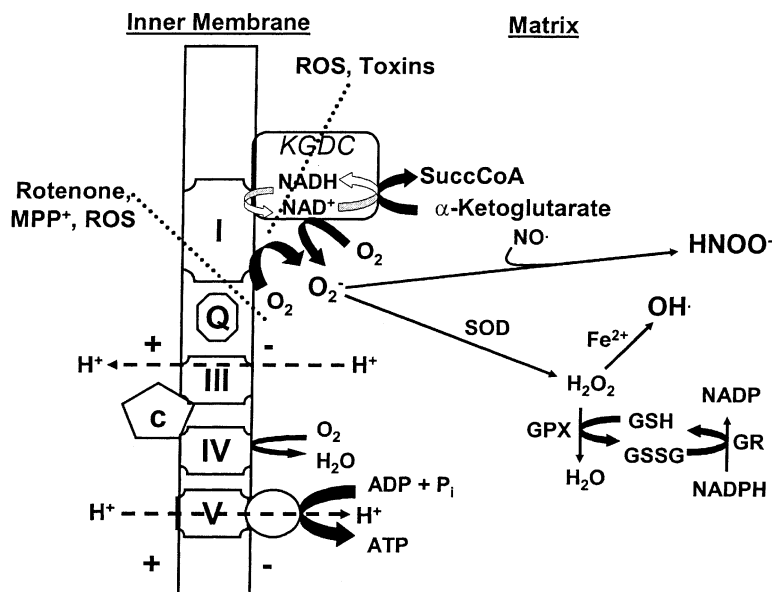


FIGURE 3. Mitochondrial generation and detoxification of reactive oxygen species. The site of mitochondrial reactive oxygen species (ROS) production most widely implicated in Parkinson's disease is complex I of the electron transport chain. Indirect evidence for involvement of complex I includes the observations that neurotoxins capable of inducing Parkinson's symptoms and neuropathology *in vivo*, such as MPP⁺ and rotenone, are inhibitors of complex I and stimulate ROS generation *in vitro*. However, these same agents result in inhibition of the overall enzyme activity of α -ketoglutarate dehydrogenase complex (α KGDC), a multisubunit complex that can also catalyze superoxide (O₂⁻) and consequently H₂O₂ production. The ROS metabolites most likely to mediate oxidative injury to mitochondria and other cellular constituents are hydroxyl radical (OH[•]) and peroxynitrite (HNOO⁻). These agents can cause oxidative damage and inhibition of mitochondrial enzyme activities, including those of complex I and α KGDC. This inhibition can lead to metabolic failure through impairment of electron transport-dependent generation of the proton-motive force that drives the synthesis of ATP at complex V (F₁F₀ ATP synthetase). Complete detoxification of superoxide depends on the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPX), and glutathione reductase (GR) together with glutathione and a sufficiently reduced redox state of NAD(P)H to drive the reduction of glutathione and consequently the reduction of H₂O₂ to H₂O.

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Our recent observations indicate that in addition to complex I, several tricarboxylic acid cycle dehydrogenases are potential sources of ROS, with α KGDH appearing the most active.³⁴ In particular, the rate of ROS production by isolated brain mitochondria measured under a state of rapid metabolism, as normally exists in neurons, was highest with α -ketoglutarate as respiratory substrate, even though several other substrates support higher rates of respiration. Zinc also promotes ROS production by the lipamide dehydrogenase component of α KGDH, but it inhibits overall

enzyme activity.³⁵ Considering the findings that environmental exposure to zinc is a risk factor for Parkinson's disease and that α KGDH enzyme activity is reduced in PD and in Alzheimer's disease,³⁶ the relationships between this enzyme complex, ROS production, and dopaminergic cell death in cellular and animal models of PD require further investigation.

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