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Forum Review

Peroxynitrite and Mitochondrial Dysfunction in the Pathogenesis of Parkinson's Disease

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ABSTRACT

Nitric oxide (NO), in excess, behaves as a cytotoxic substance mediating the pathological processes that cause neurodegeneration. The NO-induced dopaminergic cell loss causing Parkinson's disease (PD) has been postulated to include the following: an inhibition of cytochrome oxidase, ribonucleotide reductase, mitochondrial complexes I, II, and IV in the respiratory chain, superoxide dismutase, glyceraldehyde-3-phosphate dehydrogenase; activation or initiation of DNA strand breakage, poly(ADP-ribose) synthase, lipid peroxidation, and protein oxidation; release of iron; and increased generation of toxic radicals such as hydroxyl radicals and peroxynitrite. NO is formed by the conversion of L-arginine to L-citrulline by NO synthase (NOS). At least three NOS isoforms have been identified by molecular cloning and biochemical studies: a neuronal NOS or type 1 NOS (nNOS), an immunologic NOS or type 2 NOS (iNOS), and an endothelial NOS or type 3 NOS (eNOS). The enzymatic activities of eNOS or nNOS are induced by phosphorylation triggered by Ca^{2+} entering cells and binding to calmodulin. In contrast, the regulation of iNOS seems to depend on *de novo* synthesis of the enzyme in response to a variety of cytokines, such as interferon-γ and lipopolysaccharide. The evidence that NO is associated with neurotoxic processes underlying PD comes from studies using experimental models of this disease NOS inhibitors can prevent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurotoxicity. Furthermore, NO fosters dopamine depletion, and the said neurotoxicity is averted by nNOS inhibitors such as 7-nitroindazole working on tyrosine hydroxylase-immunoreactive neurons in substantia nigra pars compacta. Moreover, mutant mice lacking the nNOS gene are more resistant to MPTP neurotoxicity when compared with wild-type littermates. Selegiline, an irreversible inhibitor of monoamine oxidase B, is used in PD as a dopaminergic function-enhancing substance. Selegiline and its metabolite, desmethylselegiline, reduce apoptosis by altering the expression of a number of genes, for instance, superoxide dismutase, Bcl-2, Bcl-xl, NOS, c-Jun, and nicotinamide adenine nucleotide dehydrogenase. The selegiline-induced antiapoptotic activity is associated with prevention of a progressive reduction of mitochondrial membrane potential in preapoptotic neurons. As apoptosis is critical to the progression of neurodegenerative disease, including PD, selegiline or selegiline-like compounds to be discovered in the future may be efficacious in treating PD. *Antioxid. Redox Signal.* 5, 319–335.

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INTRODUCTION

PARKINSON'S DISEASE (PD) is the second most common neurodegenerative disorder after Alzheimer's disease, affecting ~1% of the population older than 50 years. There is a worldwide increase in the disease prevalence due to the increasing age of human populations. A definitive neuropathological diagnosis of PD requires loss of dopaminergic neurons in the substantia nigra (SN) and related brainstem nuclei, and the presence of Lewy bodies in remaining nerve cells.

The contribution of genetic factors to the pathogenesis of PD is being increasingly recognized. A point mutation that is sufficient to cause a rare autosomal dominant form of the disorder has recently been identified in the α-synuclein gene on chromosome 4 in the much more common sporadic, or "idiopathic," form of PD, and a defect of complex I of the mitochondrial respiratory chain was confirmed at the biochemical level. Disease specificity of this defect has been demonstrated for the parkinsonian SN. These findings, and the observation that the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes a Parkinson-like syndrome in humans and acts via inhibition of complex I, have triggered research interest in the mitochondrial genetics of PD.

The exact molecular mechanisms as to how perinuclear and endonuclear translocation and aggregation of α-synuclein during oxidative and nitrative stress cause Lewy body formation and progressive neurodegeneration in aging and PD remain unknown. In view of the above, we studied 1-methyl-4-phenylpyridinium (MPP^+)-induced induction, translocation, and aggregation of α-synuclein in wild-type control ($control_{wt}$) and aging mitochondrial genome knockout (RhO_{mgko}) dopaminergic (SK-N-SH) neurons. α-Synuclein expression was studied in $control_{wt}$ and aging RhO_{mgko} dopaminergic (SK-N-SH) neurons in response to the parkinsonian agent

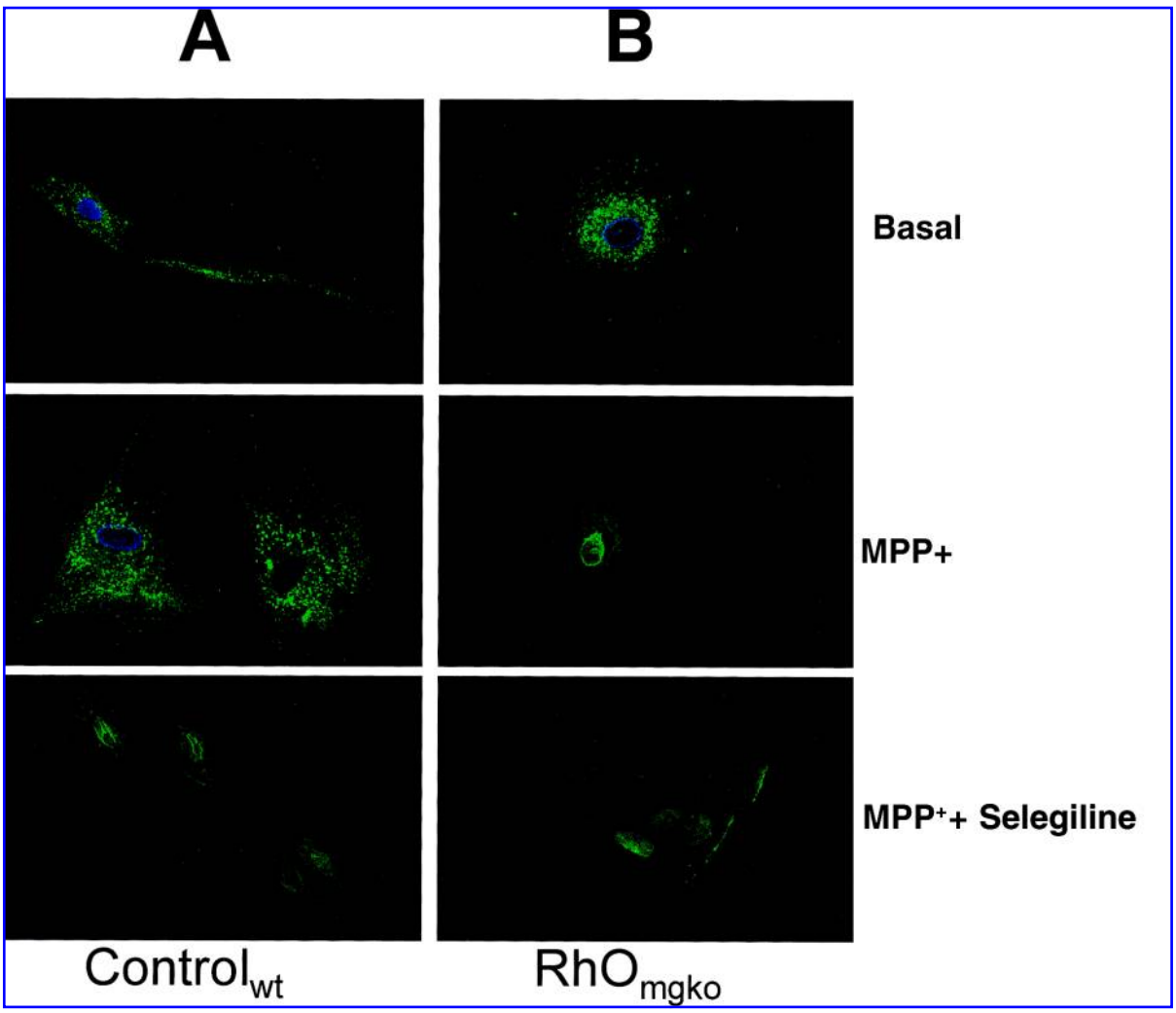


FIG. 1. α-Synuclein expression in SK-N-SH neurons. Immunofluorescence microscopic analysis of α-synuclein expression in $control_{wt}$ and aging RhO_{mgko} SK-N-SH neurons is presented. Basal expression of α-synuclein was higher in RhO_{mgko} neurons, as compared with $control_{wt}$ dopaminergic neurons. Treatment with MPP^+ enhanced perinuclear accumulation of α-synuclein in $control_{wt}$ neurons, whereas in RhO_{mgko} neurons it was localized in the endonuclear region. Pretreatment with the MAO-B inhibitor selegiline inhibited MPP^+-induced perinuclear accumulation, as well as induction of α-synuclein. Neurons were exposed to MPP^+ (1 μ*M*) and/or selegiline (10 μ*M*) overnight and immunostained with FITC-labeled anti-α-synuclein antibody (59).

MPP+ and/or selegiline. Basal α-synuclein expression was higher in RhO_{mgko} neurons as compared with $control_{wt}$ neurons. α-Synuclein expression was enhanced in response to overnight treatment with MPP+. MPP+ induced perinuclear aggregation of α-synuclein in both $control_{wt}$ and aging RhO_{mgko} neurons. In RhO_{mgko} neurons, α-synuclein was translocated in the endonuclear region. Pretreatment with selegiline suppressed perinuclear accumulation of α-synuclein in $control_{wt}$ neurons and endonuclear accumulation in aging RhO_{mgko} neurons. These observations are interpreted to suggest that parkinsonian neurotoxin, such as MPP+, can induce neurodegeneration via α-synuclein induction, translocation, and aggregation in the dopaminergic neurons (54, 59) (Fig. 1).

Glutathione (GSH) deficiency, which causes accumulation of hydrogen peroxide (H_2O_2), leads to mitochondrial damage in brain. Moreover, coenzyme Q_{10} attenuates the MPTP-induced loss of striatal dopaminergic neurons. Deficiency of striatal GSH in PD fosters oxidative stress and causes apoptosis. γ-Glutamylcysteinylglycine assists in maintaining the intracellular reducing environment, protects protein thiol groups from oxidation, and participates as a coenzyme or cofactor in a wide variety of chemical reactions. GSH exerts its antioxidant activity synergistically with both vitamin C and vitamin E. Striatal GSH deficiency in PD enhances the susceptibility of SN to destruction by endogenous or exogenous neurotoxins. Moreover, treatment with lazaroid, which inhibits lipid peroxidation, prevents death of mesencephalic dopaminergic neurons following GSH depletion (14).

The selective vulnerability and loss of certain neurons are a remarkable characteristic of age-related degenerative disorders of the brain as seen in PD. Glutamate is the major excitatory neurotransmitter in the brain, and excitotoxicity plays a role in PD. Furthermore, growing evidence implicates oxidative stress as a mediator of excitotoxic cell death. Following activation of *N*-methyl-D-aspartate (NMDA) receptors, the generation of free radicals increases, oxidation damage to lipids occurs, and antioxidants prevent cell death. Dizocilpine, which blocks NMDA receptors, may provide neuroprotection in PD (15, 16) (Fig. 2).

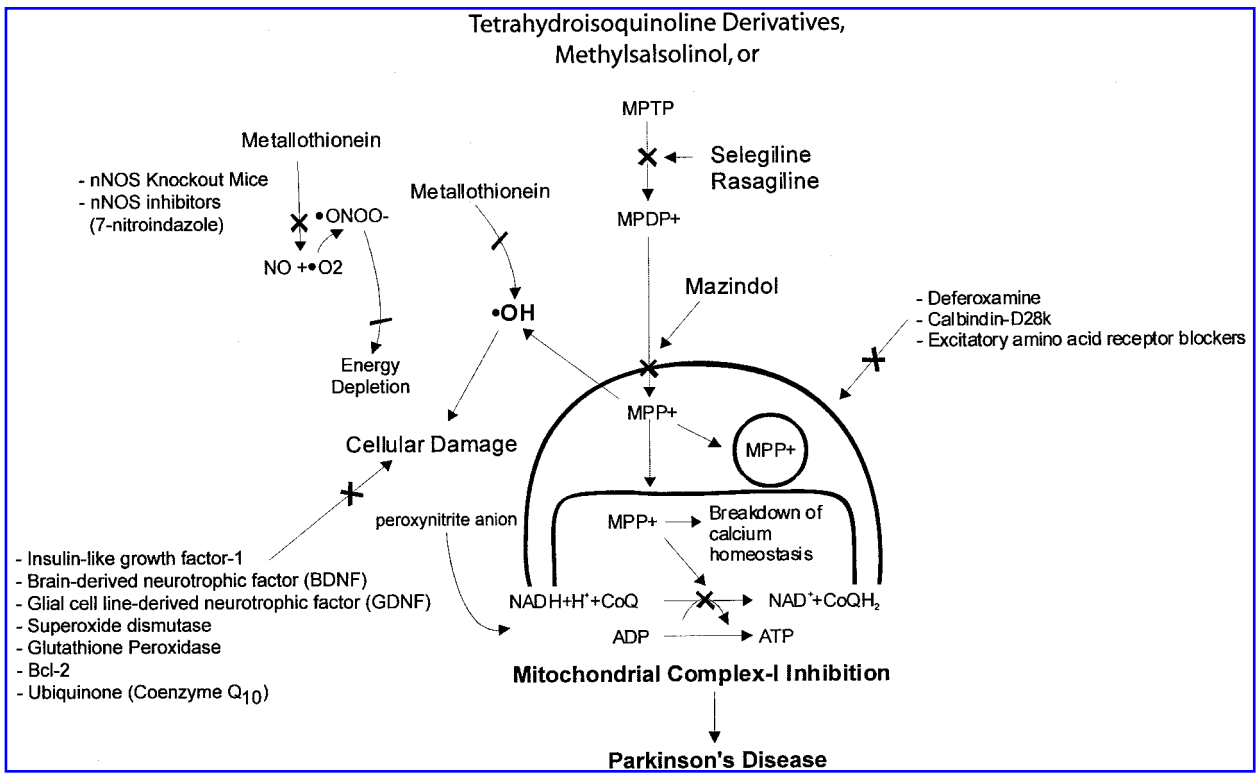


FIG. 2. Free radicals and parkinsonism. MPTP became oxidized to 1-methyl-4-phenyl-2,3-dihydropyridinium ion (MPDP+) and finally to MPP+, which generates free radicals and causes parkinsonism in human beings. A deficiency of NADH:ubiquinone oxidoreductase (EC 1.6.5.3, complex I) also causes striatal cell death. A deficiency of complex I may signify that an MPTP-like neurotoxin is generated endogenously, enhancing the vulnerability of striatum to oxidative stress reactions. The neurotoxic effects of MPP+ are blocked by metallothionein, a zinc-binding protein that scavenges ·OH, by deferoxamine, an iron-binding compound that inhibits Fenton reaction; and by *N-tert*-butyl-α-phenylnitrone, which traps free radicals. Neurons containing high concentrations of calbindin 28K are relatively resistant to MPP+. Excitatory amino acid receptor blockers such as dizocilpine attenuate MPP+-induced neurotoxicity. Amfonelic acid and mazindol, preventing the uptake of MPTP into dopaminergic neurons, and selegiline, preventing the formation of MPP+, prevent the neurotoxic effects of MPTP. After activation of excitatory amino acid receptors, there is an influx of calcium followed by activation of nNOS, which can then lead to the generation of $ONOO^-$. Consistent with such a mechanism, studies of MPTP neurotoxicity in both mice and primates have shown that inhibition of nNOS exerts neuroprotection. The toxicity of MPTP is attenuated in nNOS knockout animals and following administration of 7-NI, which inhibits NOS (31).

MITOCHONDRIAL DYSFUNCTION AND NITRIC OXIDE (NO) IN NEURODEGENERATIVE DISEASES

Damage to the mitochondrial electron transport chain has been suggested to be an important factor in the pathogenesis of a range of neurological disorders, such as PD, Alzheimer's disease, multiple sclerosis, stroke, and amyotrophic lateral sclerosis. There is also a growing body of evidence to implicate excessive or inappropriate generation of NO in these disorders. It is now well documented that NO and its toxic metabolite, peroxynitrite ($ONOO^-$), can inhibit components of the mitochondrial respiratory chain leading, if damage is severe enough, to a cellular energy deficiency state (27).

Oxidative phosphorylation consists of five protein lipid enzyme complexes located in the mitochondrial inner membrane that contain flavins (FMN, FAD), quinoid compounds (coenzyme Q_{10}), and transition metal compounds (iron-sulfur clusters, hemes, protein-bound copper). These enzymes are designated complex I (NADH:ubiquinone oxidoreductase; EC 1.6.5.3), complex II (succinate:ubiquinone oxidoreductase; EC 1.3.5.1), complex III (ubiquinol:ferrocytochrome *c* oxidoreductase; EC 1.10.2.2), complex IV (ferrocytochrome *c*:oxygen oxidoreductase or cytochrome *c* oxidase; EC 1.9.3.1), and complex V (ATP synthase; EC 3.6.1.34). A defect in mitochondrial oxidative phosphorylation, in terms of a reduction in the activity of NADH CoQ reductase (complex I), has been reported in the striatum of patients with PD. The reduction in the activity of complex I is found in the SN, but not in other areas of the brain, such as globus pallidus or cerebral cortex. Therefore, the specificity of mitochondrial impairment may play a role in the degeneration of nigrostriatal dopaminergic neurons.

This view is supported by the fact that MPTP generating MPP^+ destroys dopaminergic neurons in the SN (13, 14, 27). Lesions produced by the reversible inhibitor of succinate dehydrogenase (complex II), malonate, and the irreversible inhibitor 3-nitropropionic acid closely resemble the histologic, neurochemical, and clinical features of Huntington disease in both rats and nonhuman primates. The interruption of oxidative phosphorylation results in decreased levels of ATP. A consequence is partial neuronal depolarization and secondary activation of voltage-dependent NMDA receptors, which may result in excitotoxic neuronal cell death (secondary excitotoxicity). The increase in intracellular Ca^{2+} concentration leads to an activation of Ca^{2+}-dependent enzymes, including the constitutive neuronal nitric oxide synthase (cnNOS), which produces $NO\cdot$. $NO\cdot$ may react with the superoxide anion ($O_2^{\cdot -}$) to form $ONOO^-$. Schulz *et al.* (57) have shown that systemic administration of 7-nitroindazole (7- NI), a relatively specific inhibitor of cnNOS *in vivo,* attenuates lesions produced by striatal malonate injections or systemic treatment with 3-nitropropionic acid or MPTP. Furthermore, 7-NI attenuates increases in lactate production and hydroxyl radical ($\cdot OH$) and 3-nitrotyrosine generation *in vivo,* which may be a consequence of $ONOO^-$ formation. These results suggest that neuronal nitric oxide synthase (nNOS) inhibitors may be useful in the treatment of neurologic diseases in which excitotoxic mechanisms play a role (57).

In the CNS, NO may play important roles in neurotransmitter release, neurotransmitter reuptake, neurodevelopment, synaptic plasticity, and regulation of gene expression. However, excessive production of NO following a pathologic insult can lead to neurotoxicity. NO plays a role in mediating neurotoxicity associated with a variety of neurologic disorders, including stroke, PD, and HIV dementia (11, 53).

Due to its ability to modulate neurotransmitter release and reuptake, mitochondrial respiration, DNA synthesis, and energy metabolism, it is not surprising that NO is neurotoxic. Under conditions where NO is abnormally produced, such as when inducible nitric oxide synthase (iNOS) expression is induced in the CNS, dysregulation of normal physiologic activities of NO likely contributes to neuronal dysfunction and subsequently to neuronal death. However, acute toxicity mediated by NO appears to require production of superoxide anion.

NO in and of itself is a relatively nontoxic molecule that, in the absence of superoxide anion, will not kill cells even at extremely high concentrations. In the presence of superoxide anion, however, NO is a potent neurotoxin. The reaction of NO with superoxide anion has the fastest biochemical rate constant currently known, resulting in the formation of the potent oxidant, $ONOO^-$, which is a lipid-permeable ion with a wider range of chemical targets than NO. It can oxidize proteins, lipids, RNA, and DNA. Neurotoxicity elicited by $ONOO^-$ formation may have a dual component. $ONOO^-$ can potently inhibit mitochondrial proteins. $ONOO^-$ inhibits the function of manganese superoxide dismutase, which could lead to increased superoxide anion formation and increased $ONOO^-$ formation. Additionally, $ONOO^-$ is an effective inhibitor of enzymes in the mitochondrial respiratory chain, resulting in decreased ATP synthesis. Secondly, $ONOO^-$ efficiently modifies and breaks DNA strands and inhibits DNA ligase, which increases DNA strand breaks. DNA strand breaks activate DNA repair mechanisms. One of the initial proteins activated by DNA damage is the nuclear enzyme poly(ADP-ribose) polymerase (PARP). PARP catalyzes the attachment of ADP-ribose units from NAD to nuclear proteins such as histone and PARP itself. PARP can add hundreds of ADP-ribose units within seconds to minutes of being activated. For every mole of ADP-ribose transferred from NAD, one mole of NAD is consumed and four free-energy equivalents of ATP are required to regenerate NAD to normal cellular levels. Activation of PARP can result in a rapid drop in energy stores. If this drop is severe and sustained, it can lead to impaired cellular metabolism and ultimately cell death (11).

We (59) developed the multiple fluorescence Comet assay to examine mitochondrial, as well as nuclear, DNA damage simultaneously in a single neuron. The multiple fluorescence Comet assay is performed primarily on a single neuron using alkaline gel electrophoresis and digital fluorescence imaging microscopy. In these experiments, we have conducted the multiple fluorochrome Comet assay to determine MPP^+-induced neurotoxicity in $control_{wt}$ and aging α-synuclein-overexpressed aging RhO_{mgko} dopaminergic (SK-N-SH) neurons. Dominance of green fluorescence in $control_{wt}$ neurons suggests that in $control_{wt}$ neurons, MPP^+-induced neuronal damage remains restricted primarily to the mitochondrial region [due to synthesis of mitochondrial DNA oxidation product, 8-

hydroxy-2-deoxyguanosine (8-OH-2dG)] (Fig. 3A), whereas in α-synuclein-overexpressed aging RhO_{mgko} dopaminergic (SK-N-SH) neurons, the damage was observed in both mitochondrial and nuclear regions (Fig. 3B). These observations suggest that α-synuclein-overexpressed aging RhO_{mgko} dopaminergic (SK-N-SH) neurons are highly susceptible to MPTP-induced neurotoxicity, which could involve both mitochondrial DNA and nuclear DNA. The damage in the nuclear DNA is illustrated with red fluorescence tails due to ethidium bromide staining, and the damage to mitochondrial DNA is represented by green fluorescence tails due to fluorescein isothiocyanate (FITC)-conjugated antibody to DNA oxidation product, 8-OH-2dG (Fig. 3).

In addition to nitrite, peroxide-dependent oxidation pathways of dopamine (DA) play a major role in NO neuronal degeneration (48). Liu *et al.* (40) reported on the importance of opioids in NO-induced inflammation and neurodegeneration. Furthermore, they reported the beneficial effects of naloxone, the opioid receptor antagonist in the treatment of inflammation-related diseases. Inflammation in the brain primarily involves the participation of the two types of glial cells, microglia and astrocytes. Under physiologic conditions, microglia, the resident immune cells in the brain, serve a role of immune surveillance. Astrocytes, on the other hand, act to maintain ionic homeostasis, buffer the action of neurotransmitters, and secrete nerve growth factors. However, glia, especially microglia, readily become activated in response to immunologic challenge and injury. Activation of glia, a process termed reactive gliosis, has been observed during the pathogenesis of PD, Alzheimer's disease, multiple sclerosis, and AIDS dementia complex, as well as postneuronal death in cerebral stroke and traumatic brain injury. Activated astrocytes secrete trophic factors in an attempt to enhance neuronal survival. Activated microglia produce a variety of proinflammatory and neurotoxic factors, including the following: cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β); fatty acid metabolites, such as eicosanoids; and free radicals, such as NO and superoxide. NO and IL-1β are also produced by activated astrocytes. The production and release of these factors constitute a portion of the innate immunity that enables the host to destroy invading pathogens. However, excessive production and accumulation of these factors are deleterious to neurons, although the precise mechanism(s) and relative contribution of individual factors to neurodegeneration remain poorly understood.

Enzyme inhibitors, such as those of iNOS, are certainly valuable tools for studying the mechanism of action for inflammation-mediated neurotoxicity. However, side effects and toxicity often dampen the enthusiasm for their eventual development into agents of therapeutic value. Analysis of the modulation of the inflammatory process by physiologically relevant agents, in contrast, may prove to be a critical step in the search for clinically useful remedies. One example is the study of opioids and related compounds in the regulation of brain inflammatory process and neuroprotection. Endogenous opioid peptides represent a family of peptides that include dynorphins, enkephalins, and β-endorphins. They are widely distributed throughout the CNS, as well as in peripheral tissues such as cardiac myocytes and lymphatic cells. Through interaction with G-protein distinct coupled transmembrane opioid receptors (δ, κ, and μ), these peptides regulate a wide range of biological activities, including respiration, immune responses, and ion-channel activity. Enkephalins are capable of reducing the lipopolysaccharide (LPS)-induced IL-1β secretion from microglia. Subsequently, another group of endogenous opioid peptides, dynorphins, were found to inhibit LPS-induced NO production and to afford partial protection against LPS-induced neurotoxicity. A rather unexpected finding from these studies was the discovery that naloxone, a synthetic and nonselective opioid receptor antagonist, was capable of inhibiting LPS-induced microglial activation and production of NO and TNF-α. Most surprising of all was that (+)-naloxone, which is ineffective in blocking the stereospecific binding of opioids to their receptors, was as effective as the opioid receptor antagonist (−)-naloxone in inhibiting microglial activation and affording neuroprotection. This result suggests that the effect of naloxone isomers on microglial activation does not directly involve the opioid receptor mechanism. Analysis of the mechanism of action for naloxone isomers demonstrated that inhibition of microglial superoxide

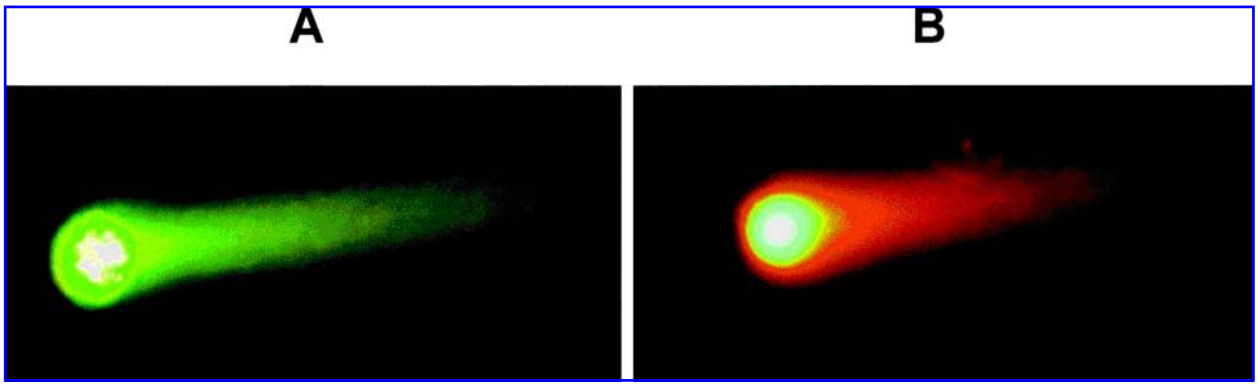


FIG. 3. 8-OH-2dG synthesis and DNA fragmentation. Multiple fluorochrome Comet assay to determine 8-OH-2dG synthesis and DNA fragmentation simultaneously in response to MPP^+ (100 μ*M*) is presented. (**A**) $Control_{wt}$ neurons. (**B**) α-Synuclein-overexpressed aging RhO_{mgko} neurons exhibited DNA damage in response to overnight exposure to MPP^+. Digital imaging was done using a SpotLite digital camera and analyzed using ImagePro computer software. Green: FITC-labeled 8-OH-2dG antibody; red: ethidium bromide-stained DNA (54, 59).

generation best correlated with their effectiveness in neuroprotection. Moreover, naloxone may have prevented the formation of $ONOO^-$ by inhibiting the production of both superoxide and NO. In a rodent model of inflammation-mediated parkinsonism, damage to dopaminergic neurons in the SN, induced by intracerebrally injected LPS, was reduced by systemically infused (−)-naloxone or (+)-naloxone. Naloxone, specifically the (+)-naloxone isomer, which does not bind conventional opioid receptors, may be a promising prototype for drugs with potential therapeutic value in the prevention and treatment of inflammation-mediated neurodegenerative disease such as PD (40).

NO IN THE PATHOGENESIS OF PD

A potential role for excitotoxic processes in PD has been strengthened by the recent observations that there appears to be a mitochondrially encoded defect in complex I activity of the electron transport chain. An impairment of oxidative phosphorylation will enhance vulnerability to excitotoxicity. SN neurons possess NMDA receptors, and there are glutamatergic inputs into the SN from both the cerebral cortex and the subthalamic nucleus (STN). After activation of excitatory amino acid receptors, there is an influx of calcium followed by activation of nNOS, which can then lead to the generation of $ONOO^-$ (see Fig. 2). Studies with MPTP-induced neurotoxicity in both mice and primates have shown that inhibition of nNOS exerts neuroprotective effects. Studies utilizing excitatory amino acid receptor antagonists have been inconsistent in mice, but show significant neuroprotective effects in primates. These results raise the prospect that excitatory amino acid antagonists for nNOS inhibitors (see Fig. 2) might be useful in the treatment of PD (11).

Current concepts of the pathogenesis of PD center on the formation of reactive oxygen species and the onset of oxidative stress leading to oxidative damage to substantia nigra pars compacta (SNPC). Extensive postmortem studies have provided evidence to support the involvement of oxidative stress in the pathogenesis of PD. In particular, these include alterations in brain iron content, impaired mitochondrial function, alterations in the antioxidant protective systems [most notably superoxide dismutase (SOD) and GSH], and evidence of oxidative damage to lipids, proteins, and DNA (13). Iron can induce oxidative stress, and intranigral injections of iron have been shown to induce a model of progressive parkinsonism. A loss of GSH is associated with incidental Lewy body disease and may represent the earliest biochemical marker of nigral cell loss. GSH depletion alone may not result in damage to nigral neurons, but may increase susceptibility to subsequent toxic or free radical exposure. The nature of the free radical species responsible for cell death in PD remains unknown, but there is evidence of involvement of ·OH, $ONOO^-$, and NO. Indeed, ·OH and $ONOO^-$ formation may be critically dependent on NO formation. Central to many of the processes involved in oxidative stress and oxidative damage in PD are the actions of monoamine oxidase-B (MAO-B). MAO-B is essential for the activation of MPTP to MPP^+, for a component of the enzymatic conversion of DA to H_2O_2, and for the activation of other potential toxins, such as isoquinolines and β-carbolines (see Fig. 2). Thus, the inhibition of MAO-B by drugs such as selegiline may protect against activation of some toxins and free radicals formed from the MAO-B oxidation of DA. In addition, selegiline may act through a mechanism unrelated to MAO-B to increase neurotrophic factor activity and up-regulate molecules such as GSH, SOD, catalase, and Bcl-2 protein, which protect against oxidant stress and apoptosis (58). Consequently, selegiline may be advantageous in the long-term treatment of PD (14). In addition to selegiline (15), propargylamine (45) may rescue or protect dopaminergic neurons in PD.

NO may play several roles in processes that lead to neurodegeneration. However, the mechanism by which NO kills cells, particularly neurons, is not fully understood. Toxicity may involve NO itself. NO inhibits a variety of enzymes, including the following: complexes I, II, and IV in the mitochondrial respiratory chain; aconitase, the citric acid cycle enzyme; ribonucleotide reductase, the rate-limiting enzyme in DNA replication; and glyceraldehyde-3-phosphate dehydrogenase in the glycolytic pathway. All these enzymes have a catalytically active nonheme iron-sulfur complex. Complex I activity is decreased in the SNPC of the PD brain and appears to be anatomically specific to the SNPC and disease-specific to PD. Because NO inhibits complex IV rather than complex I, it does not appear that NO accounts for the mitochondrial defects observed in PD. However, *in vitro* studies show that inhibition of complex IV can alter reversible mitochondrial dysfunction induced by agents that inhibit complex I, such as the 1-methyl-4-phenyl-2,3-dihydropyridinium ($MPDP^+$) ion (see Fig. 2).

It is well known that mitochondrial complex I is down-regulated during the progression of neurodegeneration in PD. Sharma *et al.* (59) attempted to delineate the possible involvement of the mitochondrial genome in neuronal repair during aging. Aging RhO_{mgko} neurons were prepared by selective inactivation of the mitochondrial DNA with 5 μg/L DNA intercalating agent, ethidium bromide, for 6–8 weeks. The $control_{wt}$ neurons exhibited structurally intact neuronal morphology, with long axons and dendrites (Fig. 4A). The aging Rho_{mgko} neurons exhibited enhanced granularity, mitochondrial aggregation, and elliptical appearance, caused by stunted neuritogenesis, down-regulation of oxidative phosphorylation, and reduced ATP generation (Fig. 4B). Neuritogenesis and ATP production in aging RhO_{mgko} neurons were regained by transfecting the aging RhO_{mgko} neurons with the mitochondrial genome encoding complex I activity (Fig. 4C). The transfection was done using pEGFP-N1 vector, Qiagen Effectine transfection reagent, and DNA enhancer as per the manufacturer's recommendations. The transfected neurons were selected using G-418, and enriched by limiting dilution technique (see Fig. 4).

There is also evidence that NO can displace iron from its binding site on ferritin, an iron-storage protein, and consequently promote lipid peroxidation. NO also can influence iron metabolism at the posttranscriptional level by interacting with cytosolic aconitase. Cytosolic aconitase has dual functions that are regulated by NO. In the absence of NO, it functions as cytosolic aconitase, but in the presence of NO it functions as the iron-responsive element binding protein to the iron-responsive element (23).

The role of NO in 6-hydroxydopamine-induced parkinsonism has been established (4). Riobo *et al.* (52) analyzed the

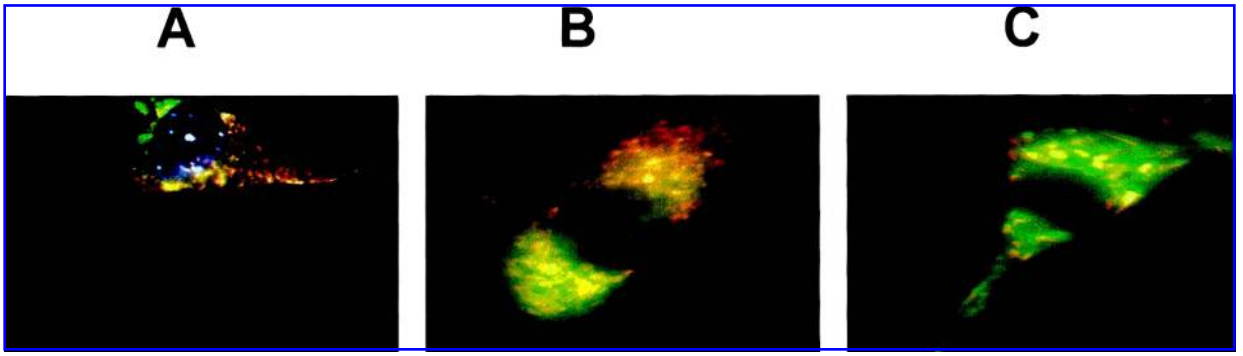


FIG. 4. Multiple fluorochrome analysis of dopaminergic (SK-N-SH) neurons. Multiple fluorochrome analysis of control$_{wt}$ (**A**), aging RhO$_{mgko}$ (**B**), and aging RhO$_{mgko}$ dopaminergic (SK-N-SH) neurons, transfected with mitochondrial genome encoding complex I activity (**C**), is presented. Aging RhO$_{mgko}$ neurons were prepared as described earlier. Fluorescence images were captured using a SpotLite digital camera and analyzed with ImagePro computer software. Selective knocking out of mitochondrial genome induced mitochondrial aggregation and changes in the neuronal morphology, represented by elliptical appearance due to reduced neuritogenesis (B). Neuritogenesis reappeared following transfection of aging RhO$_{mgko}$ neurons with mitochondrial genome (C). Red: JC-1 (molecular marker of mitochondrial membrane potential); green: FITC-conjugated MT-1; blue: nuclear stain DAPI (54, 59).

potential reaction between 6-hydroxydopamine and NO. The results showed that NO reacts with the deprotonated form of 6-hydroxydopamine at pH 7 and 37°C with a second-order rate constant of $1.5 \times 10^3\ M^{-1}\ s^{-1}$ as calculated by the rate of NO decay measured with an amperometric sensor. Accordingly, the rates of formation of 6-hydroxydopamine quinone were dependent on NO concentration. The coincubation of NO and 6-hydroxydopamine with either bovine serum albumin or α-synuclein led to tyrosine nitration of the protein, in a concentration-dependent manner and sensitive to SOD. These findings suggest the formation of $ONOO^-$ during the redox reactions following the interaction of 6-hydroxydopamine with NO. Hunot *et al.* (28), using immunohistochemistry and histochemistry, analyzed the production systems of NO in the mesencephalon of four patients with idiopathic PD and three matched control subjects. Using specific antibodies directed against the inducible isoforms of NOS, they found that this isoform was present solely in glial cells displaying the morphological characteristics of activated macrophages. Immunohistochemical analysis performed with antibodies against the neuronal isoforms of NOS, however, revealed perikarya and processes of neurons, but no glial cell staining. The number of NOS-containing cells was investigated by histoenzymology, using the NADPH-diaphorase activity of NOS. Histochemistry revealed (a) a significant increase in NADPH-diaphorase-positive glial cell density in the dopaminergic cell groups characterized by neuronal loss in PD and (b) a neuronal loss in PD that was twofold greater for pigmented NADPH-diaphorase-negative neurons than for pigmented NADPH-diaphorase-positive neurons. These data suggest a potentially deleterious role of glial cells producing excessive levels of NO in PD, which may be neurotoxic for a subpopulation of dopaminergic neurons, especially those not expressing NADPH-diaphorase activity. However, it cannot be excluded that the presence of glial cells expressing NOS in the SN of patients with PD represents a consequence of dopaminergic neuronal loss (28).

Knott *et al.* (34) examined the cellular distribution of pro- and antiinflammatory molecules in human parkinsonian and neurologically normal SN and caudate-putamen postmortem. An up-regulation of NOS- and cyclooxygenase-1- and -2-containing ameboid microglia was found in parkinsonian, but not control, nigra. Astroglia contained low levels of these molecules in both groups. Lipocortin-1-immunoreactive ameboid microglia were present within the astrocytic envelope of neurons adjacent to or within glial scars in parkinsonian nigra only. Lipocortin-1 is known to have neuroprotective and anti-inflammatory properties. Up-regulation of NOS is generally associated with neurodestruction, whereas prostaglandin synthesis may be either neurodestructive or protective. The balance of these molecules is likely to be decisive in determining neuronal survival or demise. Barthwal *et al.* (3) and Gatto *et al.* (19, 20) showed an overexpression of neutrophil nNOS in PD. Similarly, Kuiper *et al.* (35) demonstrated that the levels of L-glutamate, L-arginine, and L-citrulline were altered in cerebrospinal fluids of patients with PD, multiple system atrophy, and Alzheimer's disease. Qureshi *et al.* (51) showed that cerebrospinal fluid concentration of nitrite was elevated in PD. However, the data by Molina *et al.* (42) suggested that the plasma levels of nitrate were unrelated to the risk of developing PD.

Lo *et al.* (41) hypothesized that inhibition of NOS can prevent the destruction of dopaminergic neurons in mammals. In order to determine if NOS gene polymorphism affects the 5′-flanking region that is immediately upstream of the transcription start site lying between the TATA element and CAATT boxes in PD, and differs significantly between patients with PD and normal controls, they studied genetic polymorphism in that region of the nNOS gene in Chinese patients with PD living in Taiwan. The results indicated that the allele size distribution in that region was statistically different between patients with PD and normal subjects.

Oxidative stress is thought to be involved in the mechanism of nerve cell death in PD. Among several toxic oxidative species, NO has been proposed as a key element on the basis of the increased density of glial cells expressing iNOS in the SN of patients with PD. However, the mechanism of iNOS induction in the CNS is poorly understood, especially under

pathological conditions. Because cytokines and FcεRII/CD23 antigen have been implicated in the induction of iNOS in immune system, Hunot *et al.* (28) investigated their role in glial cells *in vitro* and in the SN of patients with PD and matched control subjects. They showed that, *in vitro,* interferon-γ together with IL-1β and TNF-α can induce the expression of CD23 in glial cells. Ligation of CD23 with specific antibodies resulted in the induction of iNOS and the subsequent release of NO. The activation of CD23 also led to an up-regulation of TNF-α production, which was dependent on NO release. In the SN of PD patients, a significant increase in the density of glial cells expressing TNF-α, IL-1β, and interferon-γ was observed. Furthermore, although CD23 was not detectable in the SN of control subjects, it was found in both astroglial and microglial cells in parkinsonian patients. These data demonstrate the existence of a cytokine/CD23-dependent activation pathway of iNOS and of proinflammatory mediators in glial cells and their involvement in the pathophysiology of PD.

Bockelmann *et al.* (6), examined postmortem putamen of PD patients and control subjects for distribution patterns of NOS-containing neurons, using the NADP-diaphorase technique. The ratio of positively stained neurons and the total number of cells (control: 1,120 ± 69 per mm^2, $n = 5$; PD: 575 ± 164 per mm^2, $n = 5$) showed striking differences between controls and PD patients. Their findings give reason to conclude that NADPH-diaphorase-positive structures may have pathogenetic importance in degenerative processes in PD putamen.

Eve *et al.* (17) studied the expression of NOS mRNA in postmortem brain in putamen, globus pallidus, and STN of neurologically normal control subjects and patients with PD using *in situ* hybridization hitochemistry. In PD, a significant increase in NOS mRNA expression was observed in the dorsal two-thirds of the STN with respect to the ventral one-third of the STN. A significant increase in NOS mRNA expression per cell in the medial medullary lamina of the globus pallidus was also observed in PD. NOS mRNA expression was significantly reduced in PD putamen. These findings provide evidence of increased activity of STN neurotransmitter systems in PD and demonstrate that basal ganglia NO systems can be selectively regulated in response to changes in dopaminergic input.

Although NO does not affect the survival of grafted dopaminergic neurons (63), depletion of GSH enhances the neurotoxic effects of NO in midbrain cultures. Under these conditions, NO triggers a programmed cell death with markers of both apoptosis and necrosis characterized by an early step of free radical production followed by a late requirement for signaling on the soluble guanylate cyclase/cyclic GMP/PKG pathway.

$ONOO^-$: A PUTATIVE CYTOTOXIN

The superoxide anion rapidly reacts with NO, yielding $ONOO^-$, and this reaction occurs *in vivo* according to the following scheme:

$$NO^{\cdot} + O_2^{-\cdot} \rightarrow ONOO^-$$

The rate constant of this reaction is near the diffusion-controlled limit ($4–7 \times 10^9\ M^{-1}\ s^{-1}$). The half-life of $ONOO^-$ at 37°C and pH 7.4 is ~1 s. $ONOO^-$ is in equilibrium with peroxynitrous acid:

$$ONOO^- + H^+ \ 7 \ ONOOH$$

The $ONOO^-$ anion itself is relatively stable, but peroxynitrous acid rapidly rearranges to form nitrate. Therefore, $ONOO^-$ is practically stable in alkaline solutions. Although it has long been thought that peroxynitrous acid decomposes to form nitrate and $^{\cdot}OH$, it is now believed that peroxynitrous acid (via an activated state: $HOONO^{\cdot}$) reacts with biological substrates in a $^{\cdot}OH$-like way. Consequently, free radicals are probably not formed during the self-decomposition of peroxynitrous acid. Nonetheless, recent evidence suggests that the reaction of $ONOO^-$ with carbon dioxide is the most important route for $ONOO^-$ in biological environments, where carbon dioxide is relatively abundant. In short, $ONOO^-$ reacts with carbon dioxide to form the nitrosoperoxycarbonate anion, which subsequently rearranges to form the nitrocarbonate anion:

$$ONOO^- + CO_2 \rightarrow ONOOCO_2^- \rightarrow O_2NOCO_2^-$$

The nitrocarbonate anion is postulated to be the proximal oxidant of $ONOO^-$-mediated reactions in biological environments. Nitrocarbonate can undergo hydrolysis, oxidize substrates via one- and two-electron transfers, and nitrosylate substrates. Carbon dioxide concentration is therefore of crucial importance for $ONOO^-$-mediated oxidation and nitrosylation. The exact biochemical fate of $ONOO^-$ in biological systems, however, is very complex and is as yet not completely clear (43).

$ONOO^-$-MEDIATED LOSS OF DOPAMINERGIC NEURONS

Involvement of NO in the destruction of nigral dopaminergic neurons in PD has not been proven, but the findings of elevated iron levels in the SNPC, impaired complex I in the SNPC, increased lipid peroxidation, and DNA damage in the nigrostriatal system implicate NO as a mediator of neuronal oxidative damage in PD, although the $^{\cdot}OH$ is also commonly associated with free radical-mediated oxidative damage.

Cellular processes with which excess NO can interact to cause dopaminergic cell death are inhibition of cytochrome *c* oxidase, ribonucleotide reductase, mitochondrial complex I, II, and IV, SOD, glyceraldehyde-3-phosphate dehydrogenase; activation or initiation of DNA strand breakage, poly(ADP-ribose) synthase, lipid peroxidation, and protein oxidation; release of iron (II); and increased generation of toxic radicals by fast reaction with the superoxide radical (17, 27).

Electron paramagnetic resonance (EPR) spectroscopy has been used to study interactions of iron proteins in cells with NO. Nitrosyl complexes such as sodium nitroprusside, which are added as experimental NO generators, themselves produce paramagnetic nitrosyl species, which may be seen by EPR. Cammack *et al.* (9) have used this to observe the effects of nitroprusside on clostridial cells. After growth in the presence of sublethal concentrations of nitroprusside, the cells have been converted into other, presumably less toxic, nitrosyl complexes, such as $(RS)_2Fe(NO)_2$. NO is cytotoxic, partly due to its effects on mitochondria. This is exploited in the de-

struction of cancer cells by the immune system. The targets include iron-sulfur proteins. It appears that species derived from NO, such as $ONOO^-$, may be responsible. Addition of $ONOO^-$ to mitochondria led to depletion of the EPR-detectable iron-sulfur clusters. Paramagnetic complexes are formed *in vivo* from hemoglobin, in conditions such as experimental endotoxic shock. This has been used to follow the course of production of NO by macrophages. Cammack *et al.* (9) have examined the effects of suppression of NOS using biopterin antagonists. Another method is to use an injected NO-trapping agent, Fe-diethyldithiocarbamate, to detect accumulated NO by EPR. In this way, they have observed the effects of depletion of serum arginine by arginase. In brains from victims of PD, a nitrosyl species identified as nitrosyl hemoglobin has been observed in SN. This is an indication for the involvement of NO or a derived species in the damage to this organ.

Discoveries over the past 10 years indicate that crucial features of neuronal communication, blood vessel modulation, and immune response are mediated by a remarkably simple chemical, NO (see 7, 24). Endogenous NO is generated from arginine by a family of three distinct calmodulin-dependent NOS enzymes. NOS from endothelial cells (eNOS) and neurons (nNOS) are both constitutively expressed enzymes, whose activities are stimulated by increases in intracellular calcium. Immune functions for NO are mediated by a calcium-independent iNOS. Expression of iNOS protein requires transcriptional activation, which is mediated by specific combinations of cytokines. All three NOS use NADPH as an electron donor and five enzyme cofactors to catalyze five-electron oxidation of arginine to NO with stoichiometric formation of citrulline.

The highest levels of NO throughout the body are found in neurons, where NO functions as a unique messenger molecule. In the autonomic nervous system, NO functions as a major nonadrenergic, noncholinergic neurotransmitter. This nonadrenergic, noncholinergic pathway plays a particularly important role in producing relaxation of smooth muscle in the cerebral circulation and the gastrointestinal, urogenital, and respiratory tracts. Dysregulation of NOS activity in autonomic nerves plays a major role in diverse pathophysiological conditions, including migraine headache. In the brain, NO functions as a neuromodulator and appears to mediate aspects of learning and memory.

Functions for NO in the brain remain less certain. Because NO is a uniquely diffusible mediator, it was proposed on theoretical grounds that NO may mediate neuronal plasticity, which underlies aspects of both development and information storage in brain. Evidence for NO involvement in synaptic plasticity has accumulated steadily. At the cellular level, NO signaling appears to be essential for two forms of neuronal plasticity: long-term potentiation in the hippocampus and long-term depression in the cerebellum.

NO appears to mediate synaptic plasticity by potentiating neurotransmitter release. In several model systems, NOS inhibitors such as nitroarginine blocked the release of neurotransmitters. In brain synaptosomes, the release of neurotransmitters evoked by stimulation of NMDA receptors is blocked by nitroarginine. Presumably, glutamate acts at NMDA receptors on NOS terminals to stimulate the formation of NO, which diffuses to adjacent terminals to enhance neurotransmitter release so that blockade of NO formation inhibits release. In addition to regulating glutamate release, NO can also regulate secretion of hormones and neuropeptides. Regulations of hormone secretion by NO has been most convincingly demonstrated in the hypothalamus (20).

MESENCEPHALIC DOPAMINERGIC NEURONS ARE RESISTANT TO CYTOTOXICITY INDUCED BY NO

Sawada *et al.* (55) investigated the intracellular mechanism that protects dopaminergic neurons against NO toxicity in rat mesencephalic cultures. $ONOO^-$, an active metabolite of NO, caused significant cytotoxic effects against dopaminergic and nondopaminergic neurons, but NO caused cytotoxic effects restricted to nondopaminergic neurons. In addition, they studied the effects of ascorbate, an antioxidant, on NO-induced neurotoxicity against dopaminergic neurons and found that coadministration of ascorbate failed to affect resistance against NO-induced neurotoxicity. These findings suggest that the protecting mechanism against NO neurotoxicity in dopaminergic neurons is based on inhibition of conversion of NO to $ONOO^-$, is dependent on the NO redox state, and is possibly due to suppression of superoxide anion production. Furthermore, they investigated NO-induced neurotoxicity with or without pretreatment with sublethal doses of MPP^+. Following pretreatment with 1 μM MPP^+, which did not show significant cytotoxic effects against dopaminergic neurons, NO demonstrated significant cytotoxicity. Therefore, MPP^+ may inhibit the protecting systems against NO neurotoxicity in dopaminergic neurons.

GLIAL CELLS, INFLAMMATORY REACTIONS, AND THE PRODUCTION OF NO

NO is involved in LPS-induced DA cell loss. Gayle *et al.* (21) characterized the effects of the proinflammatory bacteriotoxin LPS on the number of tyrosine hydroxylase-immunoreactive (THir) cells in primary mesencephalic cultures. LPS (10–80 μg/ml) selectively decreased THir cells and increased culture media levels of IL-1β and TNF-α, as well as nitrite (an index of NO production). Cultures exposed to both LPS and neutralizing antibodies to IL-1β or TNF-α showed an attenuation of the LPS-induced THir cell loss by at least 50% in both cases. Inhibition of iNOS by N^{ω}-nitro-L-arginine methyl ester (L-NAME) did not affect LPS toxicity, but increased the LPS-induced levels of both TNF-α and IL-1β. These findings suggest that neuroinflammatory stimuli that lead to elevations in cytokines may induce DA neuron cell loss in a NO-independent manner and contribute to PD pathogenesis.

NO and other reactive nitrogen species appear to play several crucial roles in the brain. These include physiological processes such as neuromodulation, neurotransmission, and synaptic plasticity, and pathological processes such as neurodegeneration and neuroinflammation. There is increasing evidence that glial cells in the CNS can produce NO *in vivo* in response to stimulation by cytokines and that this production is mediated by iNOS. Although the etiology and pathogenesis

of the major neurodegenerative and neuroinflammatory disorders (Alzheimer's disease, amyotrophic lateral sclerosis, PD, Huntington's disease, and multiple sclerosis) are unknown, numerous recent studies strongly suggest that reactive nitrogen species play an important role. Furthermore, these species are probably involved in brain damage following ischemia and reperfusion, Down's syndrome, and mitochondrial encephalopathies. Recent evidence also indicates the importance of cytoprotective proteins, such as heat shock proteins, which appear to be critically involved in protection from nitrosative and oxidative stress (8).

Perturbation of the cellular oxidant/antioxidant balance has been suggested to be involved in the neuropathogenesis of several disease states, including stroke, PD, and Alzheimer's disease, as well as "normal" physiological aging. Reactive oxygen species are constantly produced in the course of aerobic metabolism, and under normal conditions there is a steady-state balance between prooxidants and antioxidants. Most of the reactive oxygen species produced by healthy cells result from "leakage" or short circuiting of electrons at several specific locations within the cell, which then become sources of free radical production. These include the mitochondrial respiratory chain, the enzyme xanthine dehydrogenase, and, to a lesser extent, arachidonic acid metabolism and autooxidation of catecholamines or hemoproteins. However, when the rate of free radical generation exceeds the capacity of antioxidant defenses, oxidative stress ensues, causing extensive damage to DNA, proteins, and lipids.

The brain has a large potential oxidative capacity due to the high level of tissue oxygen consumption. However, the ability of the brain to withstand oxidative stress is limited because of the following anatomical, physiological, and biochemical reasons: (a) high content of easily oxidizable substrates, such as polyunsaturated fatty acids and catecholamines; (b) there are relatively low levels of antioxidants, such as GSH and vitamin E, and antioxidant enzymes, such as GSH peroxidase, catalase, and SOD; (c) the endogenous generation of reactive oxygen free radicals via several specific reactions; and (d) the elevated content of iron in specific areas of the human brain, such as globus pallidus and SN. However, cerebrospinal fluid has very little iron-binding capacity owing to its low content of transferrin. Furthermore, the CNS contains nonreplicating neuronal cells that, once damaged, may be permanently dysfunctional or committed to programmed cell death (apoptosis) (8).

$ONOO^-$ is a powerful oxidant and can nitrate aromatic amino acid residues such as tyrosine to form nitrotyrosine. Nitration to form 3-nitrotyrosine can occur on either free or protein-bound tyrosine. As the half-life of $ONOO^-$ at physiological pH is short, the detection of 3-nitrotyrosine in tissues is often used as a biological marker of $ONOO^-$ generation *in vivo*. Not only is 3-nitrotyrosine a marker for $ONOO^-$ production, it also appears that the nitration of specific proteins by $ONOO^-$ may be relevant to brain pathophysiology.

Inflammatory reaction is thought to be an important contributor to neuronal damage in neurodegenerative disorders such as Alzheimer's disease, PD, multiple sclerosis, amyotrophic lateral sclerosis, and the parkinsonism dementia complex of Guam. $ONOO^-$ is a strong oxidizing and nitrating agent, which can react with all classes of biomolecules. In the CNS, it can be generated by microglial cells activated by proinflammatory cytokines or β-amyloid peptide, and by neurons in hyperactivity of glutamate neurotransmission, mitochondrial dysfunction, and depletion of L-arginine or tetrahydrobiopterin. The first two situations correspond to cellular responses to an initial neuronal injury, and the $ONOO^-$ formed only exacerbates the inflammatory process, whereas in the third situation, the $ONOO^-$ generated directly contributes to the initiation of the neurodegenerative process (62).

$ONOO^-$, generated in inflammatory processes, is capable of nitrating and oxidizing biomolecules, implying a considerable impact on the integrity of cellular structures. Cells respond to stressful conditions by the activation of signaling pathways, including receptor tyrosine kinase-dependent pathways such as mitogen-activated protein kinases and the phosphoinositide 3-kinase/Akt pathway. $ONOO^-$ affects signaling pathways by nitration, as well as by oxidation. Whereas nitration of tyrosine residues by $ONOO^-$ modulates signaling processes relying on tyrosine phosphorylation and dephosphorylation, oxidation of phosphotyrosine phosphatases may lead to an alteration in the tyrosine phosphorylation/dephosphorylation balance. The flavanol (−)-epicatechin is a potent inhibitor of tyrosine nitration and may be used as a tool to distinguish signaling effects due to tyrosine nitration from those that are due to oxidation reactions (33).

Experimental evidence has implicated oxidative stress in the development of PD, amyotrophic lateral sclerosis, and other degenerative neuronal disorders. Recently, $ONOO^-$, which is formed by the nearly diffusion-limited reaction of NO with superoxide, has been suggested to be a mediator of oxidant-induced cellular injury. The potential role of $ONOO^-$ in the pathology of PD was evaluated by examining its effect on DOPA synthesis in PC12 pheochromocytoma cells. $ONOO^-$ was generated from the compound 3-morpholinosydnonimine (SIN-1), which releases superoxide and NO simultaneously. Exposure of PC12 cells to $ONOO^-$ for 60 min greatly diminished their ability to synthesize DOPA without apparent cell death. The inhibition was due neither to the formation of free nitrotyrosine nor to the oxidation of DOPA by $ONOO^-$. The inhibition in DOPA synthesis by SIN-1 was abolished when superoxide was scavenged by the addition of SOD. These data indicated that neither NO nor H_2O_2 generated by the dismutation of superoxide is responsible for the SIN-1-mediated inhibition of DOPA production. The inhibition of DOPA synthesis at high concentrations of SIN-1 persisted even after removal of SIN-1. The inactivation of the tyrosine hydroxylase may be responsible for the significant decline in DOPA formation by $ONOO^-$. Inactivation of tyrosine hydroxylase may be part of the initial insult in oxidative damage that eventually leads to cell death (30).

Various neurotoxins, such as 6-hyroxydopamine, salsolinol, MPP^+, and rotenone, induce mitochondrial and nuclear damage in the dopaminergic neurons. In view of the above, Ebadi and Hiramatsu (13) studied apoptosis in response to these neurotoxins in SK-N-SH neurons. All neurotoxins induced changes in neuronal morphology, represented by rounded appearance, perinuclear aggregation of mitochondria, and caspase-3 activation. These data are interpreted to suggest that these agents induce neurotoxicity primarily through caspase-3 activation and mitochondrial degeneration (Fig. 5).

Increased NO production has been implicated in many examples of neuronal injury, such as those caused by methamphetamine and MPTP to dopaminergic cells, presumably through the generation of the potent oxidant $ONOO^-$. DA is a

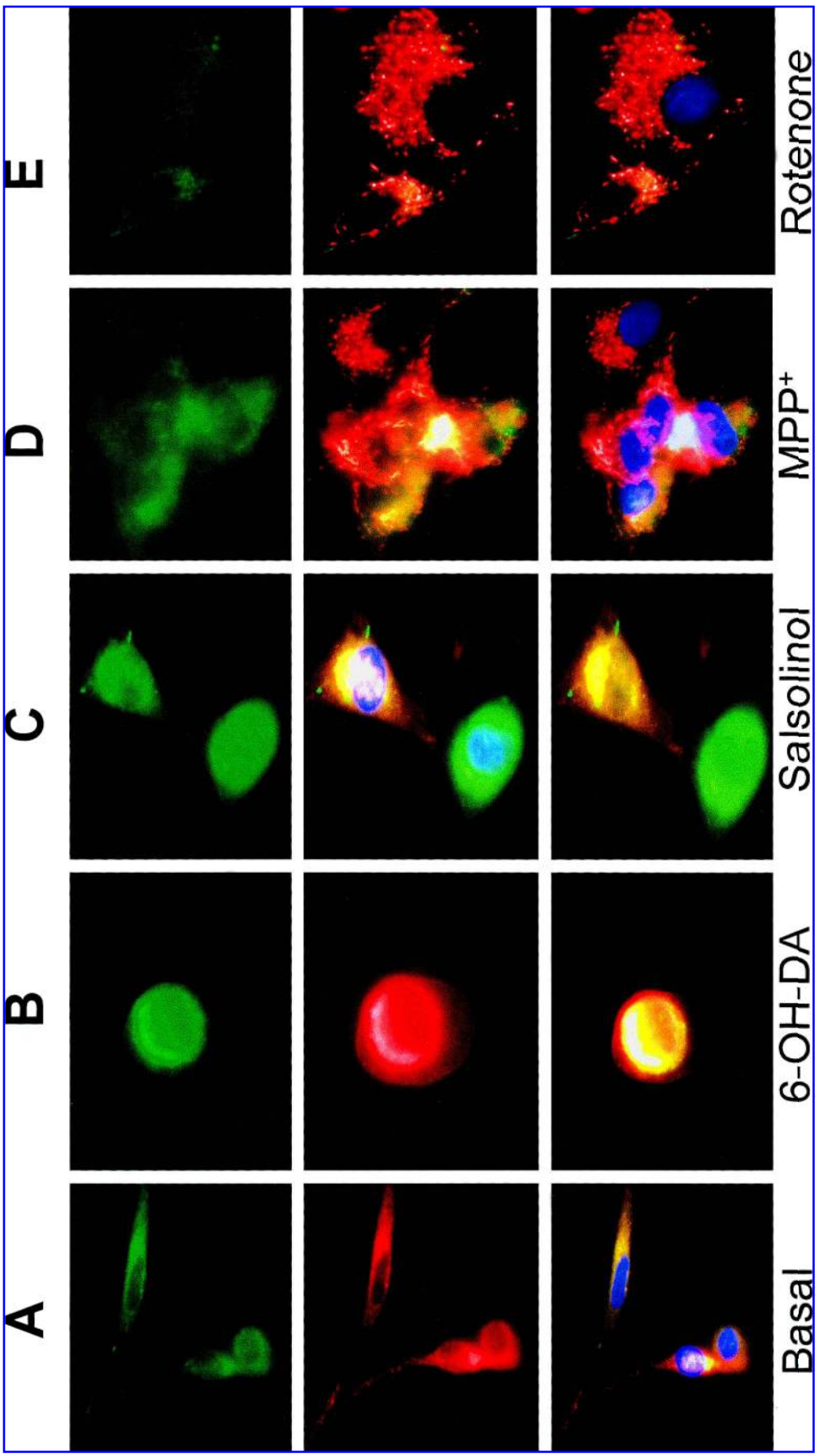


FIG. 5. Parkinsonian neurotoxins and apoptosis. Multiple fluorochrome digital fluorescence imaging microscopic analysis of apoptosis in response to various parkinsonian neurotoxins is presented. Overnight exposure to parkinsonian neurotoxins induced apoptotic changes characterized by rounded appearance, enhanced caspase-3 activation, and mitochondrial aggregation in the perinuclear region, without any typical evidence of nuclear DNA fragmentation, suggesting that early changes occur most predominantly to down-regulate the mitochondrial genome. Fluorescence images were captured by a SpotLite digital camera equipped with ImagePro computer software. Parkinsonian neurotoxins: 6-hydroxydopamine (6-OH-DA), salsolinol, and MPP^+, 100 μM each; rotenone, 100 nM. Green: FITC-conjugated anti-caspase-3; red: JC-1; blue: DAPI (36, 59).

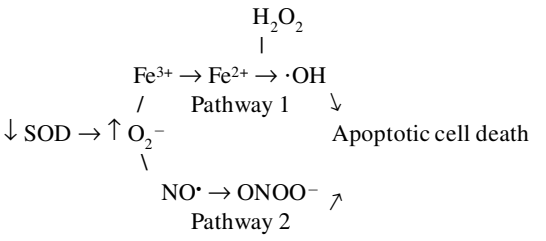
reactive molecule that, when oxidized to DA quinone, can bind to and inactivate proteins through the sulfhydryl group of the amino acid cysteine. In a study, LaVoie and Hastings (38) sought to determine if $ONOO^-$ could oxidize DA and participate in this process of protein modification. They measured the oxidation of the catecholamine by following the binding of [^{3}H]DA to the sulfhydryl-rich protein alcohol dehydrogenase. The results showed that $ONOO^-$ oxidized DA in a concentration- and pH-dependent manner. Furthermore, the resulting DA– protein conjugates were predominantly 5-cysteinyl-DA residues. In addition, it was observed that $ONOO^-$ decomposition products, such as nitrite, were also effective at oxidizing DA. These data suggest that the generation of NO and subsequent formation of $ONOO^-$ or nitrite may contribute to the selective vulnerability of dopaminergic neurons through the oxidation of DA and modification of protein (38).

Immunophilin and NOS have been implicated in the pathogenesis of PD. Araki *et al.* (1), by using a receptor autoradiographic technique, studied the sequential changes in FK-506 binding proteins, NOS, and DA uptake sites in the brain 1–8 weeks after unilateral 6-hydroxydopamine injection of the medial forebrain bundle in rats. [^{3}H]FK-506, [^{3}H]L-N^G-nitro-arginine, and [^{3}H]mazindol were used to label FK-506 binding proteins (immunophilin), NOS, and DA uptake sites, respectively. [^{3}H]FK-506 binding showed ~13–25% increase in the ipsilateral striatum 2–8 weeks after degeneration of the nigrostriatal pathway. However, no significant change in [^{3}H]FK-506 binding was observed in the ipsilateral SN during the postlesion periods. In the contralateral side, [^{3}H]FK-506 binding also showed ~13–25% increase in the striatum 2–8 weeks postlesion. The SN showed a 21% increase in [^{3}H]FK-506 binding only 2 weeks after the lesioning. On the other hand, [^{3}H]L-N^G-nitro-arginine binding showed ~21–31% increase in the parietal cortex and striatum 1–2 weeks postlesion. In the contralateral side, a 21% increase in [^{3}H]L-N^G-nitro-arginine binding was found in the dorsolateral striatum only 1 week postlesion. In contrast, degeneration of the nigrostriatal pathway caused a conspicuous loss of [^{3}H]mazindol binding in the ipsilateral striatum (87–96%), SN (36–73%), and ventral tegmental area (91–100%) during the postlesion periods. In the contralateral side, no significant changes in [^{3}H]mazindol binding were observed in these areas up to 8 weeks after the postlesion. The present study demonstrates that unilateral injection of 6-hydroxydopamine into the medial forebrain bundle of rats can cause a significant increase in [^{3}H]FK-506 and [^{3}H]L-N^G-nitro-arginine bindings in the brains. In contrast, a marked reduction in [^{3}H]mazindol binding is observed in the brains after the lesioning, indicating severe damage to the nigrostriatal dopaminergic pathway. These results suggest that immunophilin and NOS may play some role in the pathogenesis of neurodegenerative disorders such as PD.

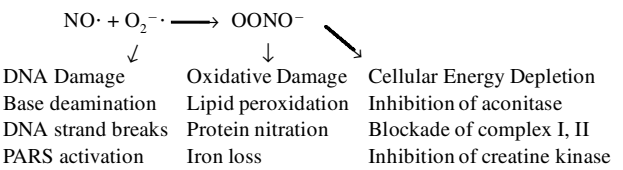
SOD PROTECTS nNOS NEURONS FROM NO-MEDIATED NEUROTOXICITY

In the nervous system, nNOS is localized in discrete populations of neurons in the cerebellum, cortex, striatum, olfactory bulb, hippocampus, basal forebrain, and brainstem. Excess production of NO via nNOS has been implicated in various neurotoxic paradigms. Excess glutamate acting via NMDA receptors may mediate cell death in focal cerebral ischemia, trauma, and epilepsy, and in neurodegenerative disease such as Huntington's disease, Alzheimer's disease, and PD.

Cu/Zn SOD is among the key cellular enzymes by which neurons and other cells detoxify free radicals and protect themselves from damage. Down-regulation of SOD causes apoptotic death of neurons. The postulated molecular mechanisms by which superoxide anions produce its toxicity are according to the following scheme:



One pathway involves superoxide purely as a reducing agent for transition metal ions such as Fe^{3+}. In this scheme, the reduced metal ion catalyzes the conversion of H_2O_2 to the highly reactive and destructive ·OH. The other pathway invokes the interaction of superoxide with NO, leading to formation of $ONOO^-$. $ONOO^-$ then can be protonated and rapidly decomposed to a strong oxidant. The toxicity of $ONOO^-$ is hereby depicted (9):



PERGOLIDE, A DA RECEPTOR AGONIST, PROVIDES PROTECTION AGAINST NO FREE RADICALS

Gómez-Vergas *et al.* (25) demonstrated that pergolide, a widely used DA agonist, has free radical scavenging and antioxidant activities. Using a direct detection system for the NO radical (NO·) by electron spin resonance (ESR) spectrometry in an *in vitro* NO·-generating system, they examined the quenching effects of pergolide on the amount of NO· generated. Pergolide dose-dependently scavenged NO·. In the competition assay, the IC_{50} value for pergolide was estimated to ~30 μ*M*. Pergolide also dose-dependently attenuated the ·OH signal in an *in vitro* $FeSO_4$-H_2O_2 ESR system with an approximate IC_{50} value of 300 μ*M*. Furthermore, this agent significantly inhibited phospholipid peroxidation of rat brain homogenates in *in vitro* experiments and after repeated administration (0.5 mg/kg/24 h, i.p. for 7 days). These findings suggest a neuroprotective role for pergolide on dopaminergic neurons due to its free radical scavenging and antioxidant properties. Similar results were found by Nishibayashi *et al.* (46).

THE ROLE OF nNOS IN MPTP-INDUCED DOPAMINERGIC NEUROTOXICITY

MPTP is used extensively in various mammalian species to produce an experimental model of PD, a common and disabling neurodegenerative disorder of unknown cause. In humans and nonhuman primates, MPTP induces irreversible and severe motor abnormalities almost identical to those observed in PD. In both monkeys and mice, MPTP replicates many of the biochemical and neuropathological changes in the nigrostriatal dopaminergic pathway found in PD. This includes a marked reduction in the levels of striatal DA and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid. In addition, as in patients with PD, animals that receive MPTP show significant reductions in the number of DA cell bodies in the SNPC. In monkeys, MPTP induces the formation of intraneuronal eosinophilic inclusions resembling Lewy bodies, a neuropathological hallmark of PD. These similarities provide appealing hints that the MPTP model may lead to important new insights into the pathogenesis of PD (50). Smith *et al.* (60) showed that NO appears to be necessary for ·OH generation in MPP^+ toxicity and may play a role in neuronal generation in PD. Przedborski *et al.* (50) injected MPTP into mice in which NOS was inhibited by 7-NI in a time- and dose-dependent fashion. 7-NI dramatically protected MPTP-injected mice against indices of severe injury to the nigrostriatal dopaminergic pathway, including reduction in striatal DA contents, decreases in numbers of nigral tyrosine hydroxylase-positive neurons, and numerous silver-stained degenerating nigral neurons (see Fig. 2). The resistance of 7-NI-injected mice to MPTP is not due to alterations in striatal pharmacokinetics or content of MPP^+, the active metabolite of MPTP. To study specifically the role of nNOS, MPTP was administered to mutant mice lacking the nNOS gene. Mutant mice are significantly more resistant to MPTP-induced neurotoxicity compared with wild-type littermates. These results indicate that neuronally derived NO mediates, in part, MPTP-induced neurotoxicity.

The similarity between the MPTP model and PD raises the possibility that NO may play a significant role in the etiology of PD. Moreover, Klivenyi *et al.* (32) showed that inhibition of nNOS protects against MPTP toxicity. Liberatore *et al.* (39) showed that after administration of MPTP to mice, there was a robust gliosis in the SNPC associated with significant up-regulation of iNOS. These changes preceded or paralleled MPTP-induced dopaminergic neurodegeneration. They also show that mutant mice lacking the iNOS gene were significantly more resistant to MPTP than their wild-type littermates. This study demonstrates that iNOS is important in the MPTP neurotoxic process and indicates that inhibitors of iNOS may provide protective benefit in the treatment of PD. Obata and Yamanaka (47) examined the effect of L-NAME, a NOS inhibitor, on extracellular potassium ion concentration ($[K^+]_o$)-enhanced ·OH generation due to MPP^+ in the rat striatum. Rats were anesthetized, and sodium salicylate in Ringer's solution (0.5 nmol/μl/min) was infused through a microdialysis probe to detect the generation of ·OH as reflected by the nonenzymatic formation of 2,3-dihydroxybenzoicacid (DHBA) in the striatum. Induction of KCl (20, 70, and 140 m*M*) increased MPP^+-induced ·OH formation trapped as DHBA in a concentration-dependent manner. However, the application of L-NAME (5 mg/kg i.v.) abolished the $[K^+]_o$-depolarization-induced ·OH formation with MPP^+. DA (10 μ*M*) also increased the levels of DHBA due to MPP^+. However, the effect of DA after application of L-NAME did not change the levels of DHBA. On the other hand, the application of allopurinol (20 mg/kg i.v. 30 min prior to study), a xanthine oxidase inhibitor, abolished both $[K^+]_o$- and DA-induced ·OH generation. Moreover, when iron (II) was administered to MPP^+ and then $[K^+]_o$ (70 m*M*)-pretreated animals, there was a marked increase in the level of DHBA. However, when corresponding experiments were performed with L-NAME-pretreated animals, the same results were obtained. Therefore, NOS activation may not be related to Fenton-type reaction via $[K^+]_o$ depolarization-induced ·OH generation. The present results suggest that $[K^+]_o$-induced depolarization augmented MPP^+-induced ·OH formation by enhancing NO synthesis. Furthermore, inhibition of nNOS prevented MPTP-induced parkinsonism in baboons (26) and in mice (12). Moreover, inhibition of nNOS by 7-NI protected against MPTP-induced neurotoxicity in mice (56).

Cutillas *et al.* (10) found that a significant loss of DA was found in rat striatal slices incubated with MPP^+ at a concentration of 2 μ*M* or higher. The addition of 7-NI, a specific inhibitor of nNOS, prevented this effect on DA when the concentration of MPP^+ was 2–5 μ*M,* but not at higher concentrations. This protection was reproduced with other less specific NOS inhibitors, such as nitroarginine and nitroarginine methyl ester. 7-NI did not protect against the DA depletion caused by the nonspecific mitochondrial chain blocker, rotenone. Neither MPP^+ nor rotenone significantly increased the nitrite concentration in striatal slices, measured as an index of NO production. The basal production of NO may be enough to trigger the DA depletion at very low concentrations of MPP^+, probably acting synergistically with cytosolic calcium increase. Higher concentrations of MPP^+ are toxic by themselves without the mediation of NO. The inhibition of nNOS may protect against DA loss at early stages of a neurodegenerative process, and it could then be considered in the treatment of neurodegenerative human processes such as PD.

Muramatsu *et al.* (44) studied the effects of the nNOS inhibitor 7-NI, nonselective NOS inhibitor L-NAME, and MAO inhibitor pargyline on MPTP-treated mice. The mice received four intraperitoneal injections of MPTP at 1-h intervals. A significant depletion in DA and DOPAC concentration was observed in the striatum from 1 day after MPTP treatment. The pretreatment of 7-NI and pargyline, but not L-NAME, dose-dependently protected MPTP-induced depletion in DA content 3 days after MPTP treatment. Their histochemical study also showed that 7-NI and pargyline can prevent a marked decrease in the nigral cells and a marked increase in astrocytes in striatum 7 days after MPTP treatment. The protective effect of 7-NI against MPTP-induced DA and DOPAC depletion in the striatum was not attenuated by intraperitoneal pretreatment with L-arginine. Furthermore, the posttreatment of 7-NI or pargyline protected against MPTP-induced depletion of DA content. These results demonstrate that the protective mechanism by which 7-NI counteracts MPTP neurotoxicity in mice may be due not only to inhibition of nNOS, but also to MAO-B inhibition. Furthermore, this study suggests that the posttreatment of 7-NI and pargyline can prevent a

significant decrease in DA levels in the striatum of MPTP-treated mice. These findings have important implications for the therapeutic time window and choice of nNOS or MAO inhibitors in patients with PD (Fig. 2).

Ferrante *et al.* (18) showed that increased nitrotyrosine immunoreactivity in SN neurons in MPTP-treated baboons is blocked by inhibition of nNOS. However, Barc *et al.* (2) demonstrated that impairment of the neuronal DA transporter activity in MPP^+-treated rat was not prevented by treatments with nNOS or PARP inhibitors.

SELENIUM DEFICIENCY ENHANCES THE EXPRESSION OF NOS

Prabhu *et al.* (49) investigated the relationship between Se status, iNOS expression, and NO production in Se-deficient and Se-supplemented RAW 264.7 macrophage cell lines. The cellular glutathione peroxidase activity, a measure of Se status, was 17-fold lower in Se-deficient RAW 264.7 cells, and the total cellular oxidative tone, as assessed by flow cytometry with 2′,7′-dichlorodihydrofluorescein diacetate, was higher in the Se-supplemented cells. Upon LPS stimulation of these cells in culture, they found significantly higher iNOS transcript and protein expression levels with an increase in NO production in Se-deficient RAW 264.7 cells than in Se-supplemented cells. Electrophoretic mobility-shift assays, nuclear factor-κB (NF-κB)-luciferase reporter assays, and western blot analyses indicate that the increased expression of iNOS in Se deficiency could be due to an increased activation and consequent nuclear localization of the redox sensitive transcription factor NF-κB. These results suggest an inverse relationship between cellular Se status and iNOS expression in LPS-stimulated RAW 264.7 cells and provide evidence for the beneficial effects of dietary Se supplementation in the prevention and/or treatment of oxidative stress-mediated inflammatory diseases.

NO-MEDIATED ERYTHROPOIETIN (EPO) PROTECTED AGAINST MPTP-INDUCED TOXICITY

EPO, produced by the kidney and fetal liver, is a cytokine-hormone that stimulates erythropoiesis under hypoxic conditions. It has been shown that EPO is produced in the CNS and its receptor is expressed on neurons. As EPO has neuroprotective effects *in vitro* and *in vivo* against brain injury (22), we investigated the effect of EPO treatment on locomotor activities of animals, survival of nigral dopaminergic neurons, and nitrate levels in SN and striatum in the MPTP-induced animal model of parkinsonism in C57/BL mice. These findings suggest that EPO has protective and treating effects in MPTP-induced neurotoxicity in this mouse model of PD via increasing NO production.

SELEGILINE AS METALLOTHIONEIN (MT) MAY PROTECT AGAINST NO TOXICITY

MT isoforms are low-molecular-weight (6,000–7,000) zinc-binding proteins containing 60–61 amino acid residues, 25–30% cysteine, and no aromatic amino acids or disulfide bonds, and binding 5–7 g of zinc/mol of protein. The mammalian MT family consists of four similar but distinct isoforms, designated as MT I–IV. MT I and MT II isoforms were first identified in the rat brain. MT III containing 68 amino acids, also known as a growth inhibitory factor, and MT IV are expressed in stratified squamous epthilia. MT isoforms are found in glial cells, as well as in neurons.

MT isoforms have been proposed to participate in the transport, accumulation, and compartmentation of zinc in various brain regions, including the areas that have extremely high concentrations of zinc, such as hippocampus. Because among its 61 amino acids, MT possesses 18–20 cysteine residues, it is the most abundant and important thiol source in the brain. In those cells that can express MT genes, they are transcriptionally regulated by metals, glucocorticoid hormones, and cytokines (14).

By using ESR spectroscopy (36, 37), we have demonstrated that rat hippocampal MT isoforms I and II were able to scavenge 1,1,-diphenyl-2-picrylhydrazyl radicals (DPPH), ·OH generated in Fenton reaction, and superoxide anions generated by the hypoxanthine and xanthine oxidase system. In addition, MT I isoform protected the isolated hepatocytes from lipid peroxidation as determined by thiobarbituric acid-bound malondialdehyde. MT antibodies scavenged DPPH radicals, ·OH, and reactive oxygen species, but not superoxide anions. The results of these studies suggest that although both isoforms of MT are able to scavenge free radicals, the MT I appears to be a superior scavenger of superoxide anions and DPPH. Moreover, antibodies formed against MT isoforms retain some, but not all, free radical scavenging actions exhibited by MT I and MT II (see Fig. 2).

Thomas *et al.* (61) postulated that neuroprotectivity of selegiline (L-deprenyl) may be unrelated to inhibiting MAO. As NO modulates activities including cerebral blood flow and memory, they examined the effect of L-deprenyl on NO. L-Deprenyl induced rapid increases in NO production in brain tissue and cerebral vessels. Vasodilation was produced by endothelial NO-dependent, as well as NO-independent, mechanisms in cerebral vessels. The drug also protected the vascular endothelium from the toxic effects of amyloid-β peptide. These novel actions of selegiline may protect neurons from ischemic or oxidative damage and suggest new therapeutic applications for L-deprenyl in vascular and neurodegenerative disease. Similar results were reported by Sharma *et al.* (59).

CONCLUSION

Damage to the mitochondrial electron transport chain has been suggested to be an important factor in the pathogenesis of a range of neurological disorders, such as PD. It is now well documented that NO and its toxic metabolite, $ONOO^-$, can inhibit components of the mitochondrial respiratory chain leading, if damage is severe enough, to a cellular energy deficiency state. Within the brain, the susceptibility of different brain cell types to NO and $ONOO^-$ exposure may be dependent on factors such as the intracellular GSH concentration and an ability to increase glycolytic flux in the face of mitochondrial damage. Thus, neurons, in contrast to astrocytes, appear particularly vulnerable to the actions of these mole-

cules. Following cytotoxin exposure, astrocytes can increase NO generation, due to *de novo* synthesis of the iNOS. Although the NO/$ONOO^-$ isoforms may not affect astrocyte survival, these molecules may diffuse out to cause mitochondrial damage, and possibly cell death, to other cells, such as neurons, in close proximity. Selegiline, selenium, coenzyme Q_{10}, naloxone, pergolide, or MT provides neuroprotection against $ONOO^-$-induced damage to dopaminergic neurons (31).

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ABBREVIATIONS

cnNOS, constitutive neuronal nitric oxide synthase; $control_{wt}$, wild-type control; DA, dopamine; DHBA, 2,3-dihydroxybenzoic acid; DOPAC, dihydroxyphenylacetic acid; DPPH, 1,1-diphenyl-2-picrylhydrazyl radicals; eNOS, endothelial nitric oxide synthase; EPO, erythropoietin; EPR, electron paramagnetic resonance; ESR, electron spin resonance; FITC, fluorescein isothiocyanate; GSH, glutathione; H_2O_2, hydrogen peroxide; IL, interleukin; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MAO, monoamine oxidase; $MPDP^+$, 1-methyl-4-phenyl-2,3-dihydropyridinium; MPP^+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MT, metallothionein; L-NAME, N^{ω}-nitro-L-arginine methyl ester; NF-κB, nuclear factor-κB; 7-NI, 7-nitroindazole; NMDA, *N*-methyl-D-aspartate; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; ·OH, hydroxyl radical; 8-OH-2dG, 8-hydroxy-2-deoxyguanosine; $ONOO^-$, peroxynitrite ion; PARP, poly(ADP-ribose) polymerase; PD, Parkinson's disease; RhO_{mgko}, mitochondrial genome knockout; SIN-1, 3-morpholinosydnonimine; SN, substantia nigra; SNPC, substantia nigra pars compacta; SOD, superoxide dismutase; STN, subthalamic nucleus; THir, tyrosine hydroxylase-immunoreactive; TNF-α, tumor necrosis factor-α.

REFERENCES

1. Araki T, Tanji H, Fujihara K, Kato H, and Itoyama Y. Increases in [^{3}H]FK-506 and [^{3}H]L-N^G-nitro-arginine binding in the rat brain after nigrostriatal dopaminergic denervation. *Metab Brain Dis* 14: 21–31, 1999.
2. Barc S, Page G, Barrier L, Piriou A, and Fauconneau B. Impairment of the neuronal dopamine transporter activity in MPP^+- treated rat was not prevented by treatments with nitric oxide synthase or poly (ADP-ribose) polymerase inhibitors. *Neurosci Lett* 314: 82–86, 2001.
3. Barthwal M, Srivastava N, Shukla R, Nag D, Seth P, Srimal R, and Dikshit M. Polymorphonuclear leukocyte nitrite content and antioxidant enzymes in Parkinson's disease patients. *Acta Neurol Scand* 100: 300–304, 1999.
4. Barthwal M, Srivastava N, and Dikshit M. Role of nitric oxide in a progressive neurodegeneration model of Parkinson's disease in the rat. *Redox Rep* 6: 297–302, 2001.
5. Beal M. Excitotoxicity and nitric oxide in Parkinson's disease pathogenesis. *Ann Neurol* 44: S110–S114, 1998.
6. Bockelmann R, Wolf G, Ransmayr G, and Riederer P. NADPH-diaphorase/nitric oxide synthase containing neurons in normal and Parkinson's disease putamen. *J Neural Transm* 7: 115–121, 1994.
7. Bredt D. Endogenous nitric oxide synthesis: biological functions and pathophysiology. *Free Radic Res* 31: 577–596, 1999.
8. Calabrese V, Bates T, and Ginffrida-Stella A. NO synthase and NO-dependent signal pathways in brain aging and neurodegenerative disorders: the role of oxidant/antioxidant balance. *Neurochem Res* 9: 1315–1341, 2000.
9. Cammack R, Shergill JK, Ananda Inalsingh V, and Hughes MN. Applications of electron paramagnetic resonance spectroscopy to study interaction of iron proteins in cells with nitric oxide. *Spectrochim Acta A Mol Biomol Spectrosc* 54: 2393–2402, 1998.
10. Cutillas B, Espejo M, and Ambrosio S. 7-Nitroindazole prevents dopamine depletion caused by low concentration of MPP^+ in rat striatal slices. *Neurochem Int* 33: 35–40, 1998.
11. Dawson V and Dawson T. Nitric oxide in neurodegeneration. *Prog Brain Res* 118: 215–229, 1998.
12. Dehmer T, Lindenau J, Haid S, Dichgans J, and Schulz J. Deficiency of inducible nitric oxide synthase protects against MPTP toxicity in vivo. *J Neurochem* 74: 2213–2216, 2002.
13. Ebadi M and Hiramatsu M. Glutathione and metallothionein in oxidative stress of Parkinson's disease. In: *Free Radicals in Brain Pathophysiology,* edited by Poli G, Cadenas E, and Packer L. New York: Marcel Dekker, Inc., 2000, pp. 427–465.
14. Ebadi M, Srinivasan SK, and Baxi M. Oxidative stress and antioxidant therapy in Parkinson's disease. *Prog Neurobiol* 48: 1–19, 1996.
15. Ebadi M, Sharma SK, Shavali S, and El Refaey H. Neuroprotective actions of selegiline. *J Neurosci Res* 67: 285–289, 2002.
16. Ebadi M, Sharma SK, Shavali S, Sangcho P, and Brekke L. Neuroprotective actions of selegiline in Parkinson's disease. *45th Annual Meeting of the Western Pharmacological Society and XXV Congreso Nacional de Farmacologia* 45: 77, 2002.
17. Eve D, Nisbet A, Kingsbury A, Hewson E, Daniel S, Lees A, Marsden C, and Foster O. Basal ganglia neuronal nitric oxide synthase mRNA expression in Parkinson's disease. *Mol Brain Res* 63: 62–71, 1998.
18. Ferrante R, Hantraye P, Brouillet E, and Beal M. Increased nitrotyrosine immunoreactivity in substantia nigra neurons in MPTP-treated baboons is blocked by inhibition of neuronal nitric oxide synthase. *Brain Res* 823: 177–182, 1999.
19. Gatto EM, Riobo N, Carreras MC, Schopfer FJ, Pargament GA, and Poderoso JJ. Circulating plasma factors in Parkinson's disease enhance nitric oxide release of normal human neutrophils. *J Neurol Sci* 165: 66–70, 1999.
20. Gatto E, Riobó N, Carreras M, Cherñavsky A, Rubio A, Satz M, and Poderoso J. Overexpression of neutrophil neuronal nitric oxide synthase in Parkinson's disease. *Nitric Oxide* 4: 534–539, 2000.

21. Gayle D, Ling Z, Tong C, Landers T, Lipton J, and Carvey P. Lipopolysaccharide (LPS)-induced dopamine cell loss in culture: roles of tumor necrosis factor-α interleukin-1β, and nitric oxide. *Dev Brain Res* 133: 27–35, 2002.
22. Genc S, Kuralay F, Genc K, Akhisaroglu M, Fadiloglu S, Yorukoglu K, Fadilolu M, and Gure A. Erythropoietin exerts neuroprotection in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated C57/BL mice via increasing nitric oxide production. *Neurosci Lett* 298: 139–141, 2001.
23. Gerlach M, Blum-Degen D, Lan J, and Riederer P. Nitric oxide in the pathogenesis of Parkinson's disease. *Adv Neurol* 80: 239–245, 1999.
24. Ghafourifar P, Bringold U, Klein S, and Richter C. Mitochondrial nitric oxide synthase, oxidative stress and apoptosis. *Biol Signals Recept* 10: 57–65, 2000.
25. Gómez-Vargas M, Nishibayashi-Asanuma S, Asanuma M, Kondo Y, Iwata E, and Ogawa N. Pergolide scavenges both hydroxyl and nitric oxide free radicals in vitro and inhibits lipid peroxidation in different regions of the rat brain. *Brain Res* 790: 202–208, 1998.
26. Hantraye P, Brouillet E, Ferrante R, Palfi S, Dolan R, Matthews R, and Beal M. Inhibition of neuronal nitric oxide synthase prevents MPTP-induced parkinsonism in baboons. *Nat Med* 2: 1017–1021, 1996.
27. Heales S, Bolaños J, Stewart V, Brookes P, Land J, and Clark J. Nitric oxide, mitochondria and neurological disease. *Biochim Biophys Acta* 1410: 215–228, 1999.
28. Hunot S, Boissiere F, Faucheux F, Brugg B, Mouatt-Prigent A, Agid Y, and Hirsch EC. Nitric oxide synthase and neuronal vulnerability in Parkinson's disease. *Neuroscience* 72: 355–363, 1996.
29. Hunot S, Dugas N, Faucheux B, Hartmann A, Tardieu M, Debré P, Agid Y, Dugas B, and Hirsch E. FcεRII/CD23 is expressed in Parkinson's disease and induces, *in vitro,* production of nitric oxide and tumor necrosis factor-α in glial cells. *J Neurosci* 19: 3440–3447, 1999.
30. Ischiropoulos H, Duran D, and Horwitz J. Peroxynitrite-mediated inhibition of DOPA synthesis in PC12 cells. *J Neurochem* 65: 2366–2372, 1995.
31. Jenner P and Olanow W. Oxidative stress and the pathogenesis of Parkinson's disease. *Neurology* 47: S161-S170, 1996.
32. Klivenyi P, Andreassen O, Ferrante R, Lancelot E, Reif D, and Beal M. Inhibition of neuronal nitric oxide synthase protects against MPTP toxicity. *Neuroreport* 11: 1265–1268, 2000.
33. Klotz LO, Schroeder P, and Sies H. Peroxynitrite signaling: receptor tyrosine kinases and activation of stress-responsive pathways. *Free Radic Biol Med* 33: 737–743, 2002.
34. Knott C, Stern G, and Wilkin GP. Inflammatory regulators in Parkinson's disease: iNOS, lipocortin-1, and cyclooxygenases-1 and –2. *Mol Cell Neurosci* 16: 724–739, 2000.
35. Kuiper MA, Teerlink T, Visser JJ, Bergmans P, Scheltens P, and Wolters E. L-glutamate, L-arginine and L-citrulline levels in cerebrospinal fluid of Parkinson's disease, multiple system atrophy, and Alzheimer's disease patients. *J Neural Transm* 107: 183–189, 2000.
36. Kumari MV, Hiramatsu M, and Ebadi M. Free radical scavenging actions of metallothionein isoforms I and II. *Free Radic Res* 29: 93–101, 1998.
37. Kumari MV, Hiramatsu M, and Ebadi M. Free radical scavenging actions of hippocampal metallothionein isoforms and of antimetallothioneins: an electron spin resonance spectroscopic study. *Cell Mol Biol* (*Noisy-le-grand*) 46: 627–636, 2000.
38. LaVoie M and Hastings T. Peroxynitrite- and nitrite-induced oxidation of dopamine: implications for nitric oxide in dopaminergic cell loss. *J Neurochem* 73: 2546–2554, 1999.
39. Liberatore G, Jackson-Lewis V, Vukosavic S, Mandir A, Vila M, McAuliffe G, Dawson V, Dawson T, and Przedborski S. Inducible nitric oxide synthase stimulates dopaminergic neurodegeneration in MPTP model of Parkinson disease. *Nat Med* 12: 1403–1409, 1999.
40. Liu B, Gao H, Wang J, Jeohn G, Cooper C, and Hong J. Role of nitric oxide in inflammation-mediated neurodegeneration. *Ann N Y Acad Sci* 962: 318–331, 2002.
41. Lo HS, Hogan EL, and Soong BW. 5′-flanking region polymorphism of the neuronal nitric oxide synthase gene with Parkinson's disease in Taiwan. *J Neurol Sci* 194: 11–13, 2002.
42. Molina J, Jiménez-Jiménez F, Navarro J, Ruiz E, Arenas J, Cabrera-Valdivia F, Vázquez A, Fernández-Calle P, Ayuso-Peralta L, Rabasa M, and Bermejo F. Plasma levels of nitrates in patients with Parkinson's disease. *J Neurol Sci* 127: 87–89, 1994.
43. Muijesers R, Folkerts G, Henricks P, Sadeghi-Hashjin G, and Nijkamp F. Peroxynitrite: a two-faced metabolite of nitric oxide. *Life Sci* 60: 1833–1845, 1997.
44. Muramatsu Y, Kurosaki R, Mikami T, Michimata M, Matsubara M, Imai Y, Kato H, Itoyama Y, and Araki T. Therapeutic effect of neuronal nitric oxide synthase inhibitor (7-nitroindazole) against MPTP neurotoxicity in mice. *Metab Brain Dis* 17: 169–182, 2002.
45. Naoi M and Maruyama W. Future of neuroprotection in Parkinson's disease. *Parkinsonism Relat Disord* 8: 139–145, 2001.
46. Nishibayashi S, Asanuma M, Kohno M, Gómez-Vargas M, and Ogawa N. Scavenging effects of dopamine agonists on nitric oxide radicals. *J Neurochem* 67: 2208–2211, 1996.
47. Obata T and Yamanaka Y. Nitric oxide enhances MPP^+-induced hydroxyl radical generation via depolarization activated nitric oxide synthase in rat striatum. *Brain Res* 902: 223–228, 2001.
48. Palumbo A, Napolitano A, Barone P, and d'Ischia M. Nitrite- and peroxide-dependent oxidation pathways of dopamine: 6-nitrodopamine and 6-hydroxydopamine formation as potential contributory mechanisms of oxidative stress- and nitric oxide-induced neurotoxicity in neuronal degeneration. *Chem Res Toxicol* 12: 1213–1222, 1999.
49. Prabhu KS, Zamamiri-Davis F, Stewart JB, Thompson JT, Sordillo LM, and Reddy CC. Selenium deficiency increases the expression of inducible nitric oxide synthase in RAW 264.7 macrophages: role of nuclear factor-kappaB in up-regulation. *Biochem J* 366: 203–209, 2002.
50. Przedborski S, Jackson-Lewis V, Yokoyama R, Shibata T, Dawson V, and Dawson T. Role of neuronal nitric oxide in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurotoxicity. *Proc Natl Acad Sci U S A* 93: 4565–4571, 1996.
51. Qureshi G, Baig S, Bednar I, Sodersten P, Forsberg G, and Siden A. Increased cerebrospinal fluid concentration of ni-

trite in Parkinson's disease. *Neuroreport* 6: 1642–1644, 1995.
52. Riobo NA, Schopfer FJ, Boveris AD, Cadenas E, and Poderoso JJ. The reaction of nitric oxide with 6-hydroxydopamine: implications for Parkinson's disease. *Free Radic Biol Med* 32: 115–121, 2002.
53. Rose S, MacKenzie GM, and Jenner P. Nitric oxide and basal ganglia degeneration. *Adv Neurol* 80: 247–257, 1999.
54. Sangchot P, Sharma S, Fugere N, Porter J, and Ebadi M. Iron-induced oxidative stress, mitochondrial aggregation, and α-synuclein translocation in SK-N-SH cells in culture. *Soc Neurosci Abstr* 28: 594–619, 2002.
55. Sawada H, Shimohama S, Kawamura T, Akaike A, Kitamura Y, Taniguchi T, and Kimura J. Mechanism of resistance to NO-induced neurotoxicity in cultured rat dopaminergic neurons. *J Neurosci Res* 46: 509–518, 1996.
56. Schulz J, Matthews R, Muqit M, Browne S, and Beal M. Inhibition of neuronal nitric oxide synthase by 7-nitroindazole protects against MPTP-induced neurotoxicity in mice. *J Neurochem* 64: 936–939, 1995.
57. Schulz J, Matthews R, Klockgether T, Dichgans J, and Beal M. The role of mitochondrial dysfunction and neuronal nitric oxide in animal models of neurodegenerative disease. *Mol Cell Biochem* 174: 193–197, 1997.
58. Sharma S, Carlson E, and Ebadi M. Selegiline mediated neuroprotection in dopaminergic neurons are mostly unrelated to its inhibition of monoamine oxidase B. *13th International Symposium on the Autonomic Nervous System Clinical Autonomic Research* 12: 307, 2002.
59. Sharma SK, Shavali S, El ReFaey H, and Ebadi M. Inhibition of α-synuclein nitration and perinuclear aggregation by antioxidants in metallothionein transgenic and aging RhO (mgko) dopaminergic neurons. *FASEB J* 16: 696–711, 2002.
60. Smith TS, Swerdlow RH, Parker WD Jr, and Bennett JP Jr. Reduction of MPP^+-induced hydroxyl radical formation and nigrostriatal MPTP toxicity by inhibiting nitric oxide synthase. *Neuroreport* 5: 2598–2600, 1994.
61. Thomas T, McLendon C, and Thomas G. L-Deprenyl: nitric oxide production and dilation of cerebral blood vessels. *Neuroreport* 9: 2595–2600, 1998.
62. Torreilles F, Salman-Tabcheh S, Guérin M, and Torreilles J. Neurodegenerative disorders: the role of peroxynitrite. *Brain Res Rev* 30: 153–163, 1999.
63. van Muiswinkel FL, Steinbusch HW, Drukarch B, and de Vente J. Identification of NO-producing and -receptive cells in mesencephalic transplants in a rat model of Parkinson's disease: a study using NADPH-*d* enzyme- and NOS_c/cGMP immunocytochemistry. *Ann N Y Acad Sci* 738: 289–304, 1994.

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2. Mirjana M. Djukic, Marina D. Jovanovic, Milica Ninkovic, Ivana Stevanovic, Katarina Ilic, Marijana Curcic, Jelena Vekic. 2012. Protective role of glutathione reductase in paraquat induced neurotoxicity. *Chemico-Biological Interactions* **199**:2, 74-86. [CrossRef]
3. L. W. Hung, V. L. Villemagne, L. Cheng, N. A. Sherratt, S. Ayton, A. R. White, P. J. Crouch, S. Lim, S. L. Leong, S. Wilkins, J. George, B. R. Roberts, C. L. L. Pham, X. Liu, F. C. K. Chiu, D. M. Shackleford, A. K. Powell, C. L. Masters, A. I. Bush, G. O'Keefe, J. G. Culvenor, R. Cappai, R. A. Cherny, P. S. Donnelly, A. F. Hill, D. I. Finkelstein, K. J. Barnham. 2012. The hypoxia imaging agent CuII(atsm) is neuroprotective and improves motor and cognitive functions in multiple animal models of Parkinson's disease. *Journal of Experimental Medicine* **209**:4, 837-854. [CrossRef]
4. Dan Lou, Xiuli Chang, Weihua Li, Qiankui Zhao, Yuzhu Wang, Zhijun Zhou. 2012. Paraquat affects the homeostasis of dopaminergic system in PC12 cells. *Pesticide Biochemistry and Physiology* . [CrossRef]
5. Ne#e Tunçel, Orhan Tansel Korkmaz, Neslihan Tekin, Erol #ener, Fahrettin Akyüz, Mine #nal. 2011. Antioxidant and Anti-Apoptotic Activity of Vasoactive Intestinal Peptide (VIP) Against 6-Hydroxy Dopamine Toxicity in the Rat Corpus Striatum. *Journal of Molecular Neuroscience* . [CrossRef]
6. Rajeswara Babu Mythri, Jayagopalan Veena, G. Harish, B. S. Shankaranarayana Rao, M. M. Srinivas Bharath. 2011. Chronic dietary supplementation with turmeric protects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-mediated neurotoxicity in vivo: implications for Parkinson's disease. *British Journal of Nutrition* **106**:01, 63-72. [CrossRef]
7. Witold Polanski, Heinz Reichmann, Gabriele Gille. 2011. Stimulation, protection and regeneration of dopaminergic neurons by 9-methyl-#-carboline: a new anti-Parkinson drug?. *Expert Review of Neurotherapeutics* **11**:6, 845-860. [CrossRef]
8. Chan Lee, Gyu Hwan Park, Jung-Hee Jang. 2011. Cellular antioxidant adaptive survival response to 6-hydroxydopamine-induced nitrosative cell death in C6 glioma cells. *Toxicology* **283**:2-3, 118-128. [CrossRef]
9. James Leiper, Manasi Nandi. 2011. The therapeutic potential of targeting endogenous inhibitors of nitric oxide synthesis. *Nature Reviews Drug Discovery* **10**:4, 277-291. [CrossRef]
10. David J. Graber, Paul J. Park, William F. Hickey, Brent T. Harris. 2011. Synthetic Triterpenoid CDDO Derivatives Modulate Cytoprotective or Immunological Properties in Astrocytes, Neurons, and Microglia. *Journal of Neuroimmune Pharmacology* **6**:1, 107-120. [CrossRef]
11. M. Obulesu, R. Venu, R. Somashekhar. 2011. Lipid peroxidation in Alzheimer’s Disease: emphasis on metal-mediated neurotoxicity. *Acta Neurologica Scandinavica* no-no. [CrossRef]
12. Micronutrients for the Prevention and Improvement of the Standard Therapy for Parkinson’s Disease 197-220. [CrossRef]
13. Ruth A. Roberts, Robert A. Smith, Stephen Safe, Csaba Szabo, Ronald B. Tjalkens, Fredika M. Robertson. 2010. Toxicological and pathophysiological roles of reactive oxygen and nitrogen species. *Toxicology* **276**:2, 85-94. [CrossRef]
14. Agata Adamczyk, Anna Ka#mierczak, Grzegorz Arkadiusz Czapski, Joanna Benigna Strosznajder. 2010. #-Synuclein induced cell death in mouse hippocampal (HT22) cells is mediated by nitric oxide-dependent activation of caspase-3. *FEBS Letters* **584**:15, 3504-3508. [CrossRef]
15. Andreas Daiber. 2010. Redox signaling (cross-talk) from and to mitochondria involves mitochondrial pores and reactive oxygen species. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1797**:6-7, 897-906. [CrossRef]
16. Xi-lin Lu, Xiao-li Yao, Zhiyong Liu, Heng Zhang, Wei Li, Zhenxing Li, Guan-Lei Wang, Jiyan Pang, Yongcheng Lin, Zhongliang Xu, Ling Chen, Zhong Pei, Jinsheng Zeng. 2010. Protective effects of

xyloketal B against MPP+-induced neurotoxicity in Caenorhabditis elegans and PC12 cells. *Brain Research* **1332**, 110-119. [CrossRef]

17. Yunjing LUO, Jinglin PAN, Yanshu PAN, Zhiguo HAN, Rugang ZHONG. 2010. Evaluation of the Protective Effects of Chinese Herbs against Biomolecule Damage Induced by Peroxynitrite. *Bioscience, Biotechnology, and Biochemistry* **74**:7, 1350-1354. [CrossRef]
18. Kuzhuvelil B, Sanjit Dey, Bharat AggarwalPrevention and Treatment of Neurodegenerative Diseases by Spice-Derived Phytochemicals **20091218**, . [CrossRef]
19. Miguel A. Ortiz-Ortiz, José M. Morán, Rosa A. González-Polo, Mireia Niso-Santano, Germán Soler, José M. Bravo-San Pedro, José M. Fuentes. 2009. Nitric Oxide-Mediated Toxicity in Paraquat-Exposed SH-SY5Y Cells: A Protective Role of 7-Nitroindazole. *Neurotoxicity Research* **16**:2, 160-173. [CrossRef]
20. Tzu-Hurng Cheng, Jeremy J-W Chen, Neng-Lang Shih, Jia-Wei Lin, Ju-Chi Liu, Yen-Ling Chen, Cheng-Hsien Chen, Jin-Jer Chen. 2009. MECHANICAL STRETCH INDUCES ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE EXPRESSION IN NEONATAL RAT CARDIOMYOCYTES. *Clinical and Experimental Pharmacology and Physiology* **36**:5-6, 559-566. [CrossRef]
21. Tetsuya Konishi. 2009. Brain Oxidative Stress as Basic Target of Antioxidant Traditional Oriental Medicines. *Neurochemical Research* **34**:4, 711-716. [CrossRef]
22. Zhenquan Jia, Hong Zhu, Michael J. Vitto, Bhaba R. Misra, Yunbo Li, Hara P. Misra. 2009. Alpha-lipoic acid potently inhibits peroxynitrite-mediated DNA strand breakage and hydroxyl radical formation: implications for the neuroprotective effects of alpha-lipoic acid. *Molecular and Cellular Biochemistry* **323**:1-2, 131-138. [CrossRef]
23. Ilona Joniec, Agnieszka Ciesielska, Iwona Kurkowska-Jastrzebska, Adam Przybylkowski, Anna Czlonkowska, Andrzej Czlonkowski. 2009. Age- and sex-differences in the nitric oxide synthase expression and dopamine concentration in the murine model of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Brain Research* **1261**, 7-19. [CrossRef]
24. Rebecca L. Miller, Marilyn James-Kracke, Grace Y. Sun, Albert Y. Sun. 2009. Oxidative and Inflammatory Pathways in Parkinson's Disease. *Neurochemical Research* **34**:1, 55-65. [CrossRef]
25. Dana B. Hancock, Eden R. Martin, Jeffery M. Vance, William K. Scott. 2008. Nitric oxide synthase genes and their interactions with environmental factors in Parkinson's disease. *Neurogenetics* **9**:4, 249-262. [CrossRef]
26. Clara De Palma, Sestina Falcone, Chris Panzeri, Sonia Radice, Maria Teresa Bassi, Emilio Clementi. 2008. Endothelial nitric oxide synthase overexpression by neuronal cells in neurodegeneration: a link between inflammation and neuroprotection. *Journal of Neurochemistry* **106**:1, 193-204. [CrossRef]
27. John P. Seibyl. 2008. Single-Photon Emission Computed Tomography and Positron Emission Tomography Evaluations of Patients With Central Motor Disorders. *Seminars in Nuclear Medicine* **38**:4, 274-286. [CrossRef]
28. K KIM. 2008. Differential expression of neuronal and inducible nitric oxide synthase in rat brain after subchronic administration of 3-monochloro-1,2-propanediol. *Food and Chemical Toxicology* **46**:3, 955-960. [CrossRef]
29. Je-Seong Won, Avtar K. Singh, Inderjit Singh. 2007. Lactosylceramide: a lipid second messenger in neuroinflammatory disease. *Journal of Neurochemistry* **103**:s1, 180-191. [CrossRef]
30. O. A. Sergeeva, N. Doreulee, A. N. Chepkova, T. Kazmierczak, H. L. Haas. 2007. Long-term depression of cortico-striatal synaptic transmission by DHPG depends on endocannabinoid release and nitric oxide synthesis. *European Journal of Neuroscience* **26**:7, 1889-1894. [CrossRef]
31. J WANG, Z XU, H FANG, H DUHART, T PATTERSON, S ALI. 2007. Gene expression profiling of MPP+-treated MN9D cells: A mechanism of toxicity study. *NeuroToxicology* **28**:5, 979-987. [CrossRef]

32. Tobias Jung, Martina Engels, Lars-Oliver Klotz, Klaus-Dietrich Kröncke, Tilman Grune. 2007. Nitrotyrosine and protein carbonyls are equally distributed in HT22 cells after nitrosative stress. *Free Radical Biology and Medicine* **42**:6, 773-786. [CrossRef]
33. R DINISOLIVEIRA, F REMIAO, H CARMO, J DUARTE, A NAVARRO, M BASTOS, F CARVALHO. 2006. Paraquat exposure as an etiological factor of Parkinson's disease. *NeuroToxicology* **27**:6, 1110-1122. [CrossRef]
34. Ramkumar Kavya, Rohit Saluja, Sarika Singh, Madhu Dikshit. 2006. Nitric oxide synthase regulation and diversity: Implications in Parkinson's disease#. *Nitric Oxide* **15**:4, 280-294. [CrossRef]
35. Dana B. Hancock, Eden R. Martin, Kenichiro Fujiwara, Mark A. Stacy, Burton L. Scott, Jeffrey M. Stajich, Rita Jewett, Yi-Ju Li, Michael A. Hauser, Jeffery M. Vance, William K. Scott. 2006. NOS2A and the modulating effect of cigarette smoking in Parkinson's disease. *Annals of Neurology* **60**:3, 366-373. [CrossRef]
36. Christine Winter, Kai Hosmann, Daniel Harnack, Wassilios Meissner, Gesine Paul, Rudolf Morgenstern, Andreas Kupsch. 2006. Subthalamic nucleus lesioning inhibits expression and phosphorylation of c-Jun in nigral neurons in the rat's 6-OHDA model of Parkinson's disease. *Synapse* **60**:1, 69-80. [CrossRef]
37. S SHARMA, H ELREFAEY, M EBADI. 2006. Complex-1 activity and 18F-DOPA uptake in genetically engineered mouse model of Parkinson's disease and the neuroprotective role of coenzyme Q10. *Brain Research Bulletin* **70**:1, 22-32. [CrossRef]
38. Sergey Kalinin , Paul E. Polak , Jose L. M. Madrigal , Vitaliy Gavrilyuk , Anthony Sharp , Neelima Chauhan , Marc Marien , Francis Colpaert , Dr. Douglas L. Feinstein . 2006. Beta-Amyloid-Dependent Expression of NOS2in Neurons: Prevention by an #2-Adrenergic Antagonist. *Antioxidants & Redox Signaling* **8**:5-6, 873-833. [Abstract] [Full Text PDF] [Full Text PDF with Links]
39. Vilmante Borutaite, Guy C. Brown. 2006. S-nitrosothiol inhibition of mitochondrial complex I causes a reversible increase in mitochondrial hydrogen peroxide production. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1757**:5-6, 562-566. [CrossRef]
40. Guo-Hua Fan, Hai-Yan Zhou, Hui Yang, Sheng-Di Chen. 2006. Heat shock proteins reduce #-synuclein aggregation induced by MPP+ in SK-N-SH cells. *FEBS Letters* **580**:13, 3091-3098. [CrossRef]
41. Douglas A. Richards, Michael A. Silva, Adam J. Devall. 2006. Electrochemical detection of free 3-nitrotyrosine: Application to microdialysis studies. *Analytical Biochemistry* **351**:1, 77-83. [CrossRef]
42. Sirirat Klongpanichapak, Piyarat Govitrapong, Sushil K. Sharma, Manuchair Ebadi. 2006. Attenuation of Cocaine and Methamphetamine Neurotoxicity by Coenzyme Q10. *Neurochemical Research* **31**:3, 303-311. [CrossRef]
43. Shaik Shavali, Colin K. Combs, Manuchair Ebadi. 2006. Reactive Macrophages Increase Oxidative Stress and Alpha-Synuclein Nitration During Death of Dopaminergic Neuronal Cells in Co-Culture: Relevance to Parkinson's Disease. *Neurochemical Research* **31**:1, 85-94. [CrossRef]
44. Manuchair Ebadi, Sushil Sharma, Sawitri Wanpen, Shaik Shavali, Hesham El ReFaeyCoenzyme Q10 Stabilizes Mitochondria in Parkinson's Disease **20055781**, 127-153. [CrossRef]
45. Tracy T. Cao, Lei Ma, Geeta Kandpal, Lee Warren, J. Fred Hess, Guy R. Seabrook. 2005. Increased nuclear factor-erythroid 2 p45-related factor 2 activity protects SH-SY5Y cells against oxidative damage. *Journal of Neurochemistry* **95**:2, 406-417. [CrossRef]
46. A KANTHASAMY, M KITAZAWA, A KANTHASAMY, V ANANTHARAM. 2005. Dieldrin-Induced Neurotoxicity: Relevance to Parkinson's Disease Pathogenesis. *NeuroToxicology* **26**:4, 701-719. [CrossRef]
47. V. A. Sukhanov, I. D. Ionov, L. A. Piruzyan. 2005. Neurodegenerative Disorders: The Role of Genetic Factors in Their Origin and the Efficiency of Treatment. *Human Physiology* **31**:4, 472-482. [CrossRef]
48. F.X. Guix, I. Uribesalgo, M. Coma, F.J. Muñoz. 2005. The physiology and pathophysiology of nitric oxide in the brain. *Progress in Neurobiology* **76**:2, 126-152. [CrossRef]

49. Yong Yang, Xing Liu, Yan Long, Fang Wang, Jian-Hua Ding, Su-Yi Liu, Ye-Hong Sun, Hong-Hong Yao, Hai Wang, Jie Wu, Gang Hu. 2005. Systematic administration of iptakalim, an ATP-sensitive potassium channel opener, prevents rotenone-induced motor and neurochemical alterations in rats. *Journal of Neuroscience Research* **80**:3, 442-449. [CrossRef]
50. Jaturaporn Chagkutip, Piyarat Govitrapong, Sirirat Klongpanichpak, Manuchair Ebadi. 2005. Mechanism of 1-Methyl-4-Phenylpyridinium-Induced Dopamine Release from PC12 Cells. *Neurochemical Research* **30**:5, 633-639. [CrossRef]
51. Eugenio Mocchegiani, Carlo Bertoni-Freddari, Fiorella Marcellini, Marco Malavolta. 2005. Brain, aging and neurodegeneration: Role of zinc ion availability. *Progress in Neurobiology* **75**:6, 367-390. [CrossRef]
52. Charles B. Glaser, Ghiam Yamin, Vladimir N. Uversky, Anthony L. Fink. 2005. Methionine oxidation, #-synuclein and Parkinson's disease. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* **1703**:2, 157-169. [CrossRef]
53. Andrea Ernst, Alexandra Stolzing, Grit Sandig, Tilman Grune. 2004. Antioxidants effectively prevent oxidation-induced protein damage in OLN 93 cells. *Archives of Biochemistry and Biophysics* **421**:1, 54-60. [CrossRef]
54. J Chagkutip. 2003. 1-Methyl-4-phenylpyridinium-induced down-regulation of dopamine transporter function correlates with a reduction in dopamine transporter cell surface expression. *Biochemical and Biophysical Research Communications* **311**:1, 49-54. [CrossRef]
55. Pedram Ghafourifar , Carol A. Colton . 2003. Compartmentalized Nitrosation and Nitration in Mitochondria. *Antioxidants & Redox Signaling* **5**:3, 349-354. [Abstract] [Full Text PDF] [Full Text PDF with Links]
56. Pedram Ghafourifar , Carol A. Colton . 2003. Mitochondria and Nitric Oxide. *Antioxidants & Redox Signaling* **5**:3, 249-250. [Citation] [Full Text PDF] [Full Text PDF with Links]