Environment, Mitochondria, and Parkinson's Disease

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Parkinson's disease (PD) is a common and disabling neurodegenerative disease marked by progressive motor dysfunction, which results from selective degeneration of the nigrostriatal pathway. Epidemiological studies indicate that exposure to pesticides, rural living, farming, and drinking well water are associated with an increased risk of developing PD. Rare cases of PD are caused by mutations in nuclear genes, and there is increasing evidence for susceptibility genes that alter disease risk. Parkinson's disease is also associated with a systemic defect in mitochondrial complex I activity. Animal models indicate that exposure to inhibitors of mitochondrial complex I, including pesticides, is sufficient to reproduce the features of PD, but genetic factors clearly modulate susceptibility. Complex I defects may result in oxidative stress and increase the susceptibility of neurons to excitotoxic death. In this way, environmental exposures and mitochondrial dysfunction may interact and result in neurodegeneration. NEUROSCIENTIST 8(3):192–197, 2002

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Parkinson's disease (PD) is a late-onset neurodegenerative disease, characterized clinically by tremor, rigidity, and bradykinesia (slowness of movement). Additional signs and symptoms may include gait and balance difficulties and autonomic dysfunction. About one-third of people with PD develop dementia. Aging is a major risk factor for PD; it is uncommon before age 40, but its incidence increases progressively thereafter.

Pathologically, PD is marked by progressive, selective degeneration of the nigrostriatal dopaminergic pathway. Although there is some involvement of other dopaminergic cell groups, such as the ventral tegmental area, and other catecholamine-containing cells, such as the locus ceruleus, the cells of the substantia nigra pars compacta bear the brunt of the damage in PD. It is not clear whether degeneration begins in the nerve terminals or the cell bodies of these neurons. Many degenerating dopaminergic cells contain round, cytoplasmic inclusions called Lewy bodies (Mirra and others 1997). By electron microscopy, these inclusions have a dense amorphous core surrounded by filamentous material. Lewy bodies contain the widely expressed synaptic protein, α-synuclein, and polyubiquitin (Mirra and others 1997; Spillantini and others 1997).

The role of genetics in typical PD is a matter of debate. Rarely, patients have a familial form of PD, usually with an earlier disease onset and often with atypical clinical features. Such familial cases of PD have been associated with mutations in proteins such as α-synuclein and parkin (Polymeropoulos and others 1997; Kitada and others 1998). However, most cases of typical PD (sporadic PD) are characterized by little, if any, family history, and disease onset late in life. Although the causes of sporadic PD are unknown, it is widely believed that a combination of genetic susceptibilities and environmental exposures account for most cases. Despite this widespread belief, the specific genetic and environmental factors important in PD remain elusive.

Environment and PD

Numerous epidemiological studies have been performed in an attempt to define factors associated with an increased risk of developing PD. Although no specific causative smoking gun has been identified definitively, there are intriguing clues. For example, case-control studies have suggested that i) rural living, ii) farming as an occupation, iii) drinking well water, and iv) pesticide exposure are each associated with an increased risk of PD. Although not all studies have been positive, recent meta-analyses have indicated that the risks associated with these factors are likely to be real (Priyadarshi and others 2000, 2001). The extent to which these factors are related or independent is not clear, however. An additional environmental risk for PD is occupational exposure to certain metals, most notably manganese (Gorell and others 1999). Interestingly, many pesticides and manganese share the common mechanism of causing mitochondrial dysfunction. Such mechanistic commonalities may eventually provide insights into PD pathogenesis.

Unfortunately, with the exception of 1-methyl-4-1,2,3,6-tetrahydropyridine (MPTP), which will be discussed below, no specific environmental agent has been linked conclusively to PD pathogenesis. There are several possible explanations for this. First, chronic low-level environmental exposures may be more relevant to sporadic PD but may be very

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difficult to detect. Second, acute environmental exposures may produce delayed or slowly progressive degeneration, so that the disease expression (symptom onset) might be remote in time and place from exposure. Indeed, imaging and postmortem studies suggest that exposure to MPTP in the remote past may cause a progressive parkinsonian disorder (Vingerhoets and others 1994; Langston and others 1999). Third, individual genetic variations may explain the development of PD only in a subset of individuals exposed to similar toxins. Such genetic variation could involve polymorphic variations in disease-associated genes, such as α-synuclein (Farrer and others 2001), xenobiotic metabolism (Menegon and others 1998), mitochondrial function (Schapira 1998), or even blood-brain-barrier function.

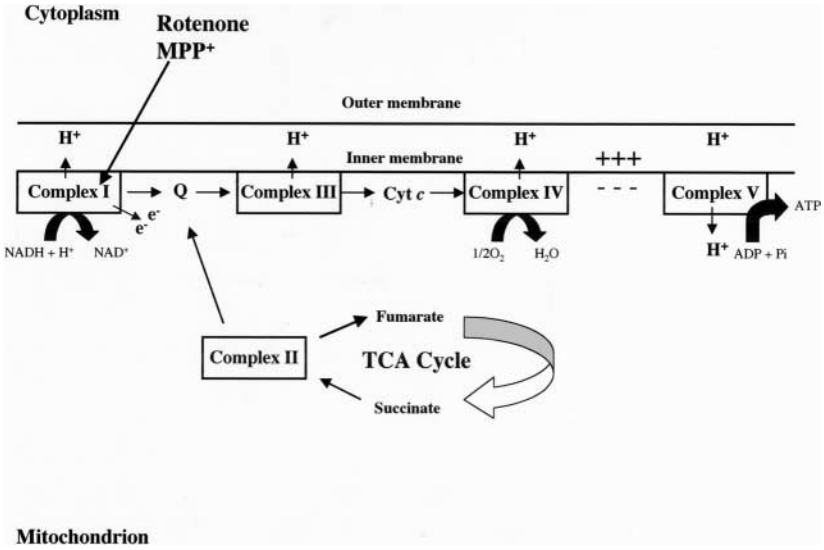

Fig. 1. Schematic diagram of the mitochondrial electron transport chain. The electron transport chain consists of five enzyme complexes that create a proton gradient and undergo oxidation-reduction reactions. This proton gradient is used to synthesize ATP. Rotenone and MPP^+ act by inhibiting complex I.

Environment, Mitochondria, and PD

In 1982, the product of a botched meperidine synthesis, MPTP, was injected inadvertently by several drug addicts in the San Francisco bay area. This unfortunate event provided direct evidence for the potential role of "environmental" toxins in PD pathogenesis: MPTP caused an acute and permanent parkinsonian syndrome in these individuals (Langston and others 1983). Investigations of the mechanisms through which MPTP exposure resulted in selective dopaminergic cell death have uncovered clues to PD pathogenesis. 1-Methyl-4-phenylpyridinium ion (MPP^+), the active metabolite of MPTP, is a substrate for the dopamine transporter, which is selectively expressed in dopaminergic neurons (Javitch and Snyder 1984). Once inside these neurons, MPP^+ accumulates in mitochondria and exerts its toxicity by inhibiting complex I (Fig. 1) of the mitochondrial electron transport chain (Nicklas and others 1985). Importantly, this finding suggested that mitochondrial dysfunction may have a role in PD pathogenesis. Thus, the epidemiological study that led to the identification of MPTP ultimately suggested a pathogenic mechanism.

With an understanding of the mechanism of action of MPTP, several laboratories began to investigate the status of mitochondrial complex I in sporadic PD. These studies demonstrated that PD patients express modest, but reproducible, reductions in complex I activity in tissues including brain and platelets (Mizuno and others 1989; Parker and others 1989; Schapira and others 1989). On average, in platelets, there appears to be about a 25% decrease in complex I activity, but current complex I assays are insensitive to subtle defects (Greenamyre and others 2001). Thus, these results suggest that PD patients have a *systemic* complex I defect, affecting both brain and peripheral tissues.

The use of cytoplasmic hybrid (cybrid) cells has suggested that PD patients may harbor mutations in mitochondrially encoded subunits of complex I. Cybrids are created when cells devoid of mtDNA (due to long-term, low-dose exposure to ethidium bromide) are repopulated with mtDNA from platelets of PD patients. These cybrids express mtDNA from PD patients, or age-matched controls, on a uniform nuclear background. This technique has demonstrated that the reduced complex I activity seen in PD platelets can be transmitted stably into cybrid cell lines, which suggests that it may result from mutations in mtDNA (Gu and others 1998; Swerdlow and others 1996). On the other hand, despite intensive efforts, no causative mtDNA mutations have been demonstrated unambiguously.

In summary, PD is associated with a modest, systemic defect in complex I activity, which may result from genetic or acquired alterations in mitochondrial protein subunits, or environmental exposures that inhibit complex I function (Schapira 1998; Le Couteur and others 1999). Whether or not this complex I abnormality had anything to do with PD pathogenesis, however, remained uncertain. That is, could a mild, systemic mitochondrial defect cause such exquisitely selective neurodegeneration?

Rotenone—Proof of Principle

To address this question, we used rotenone to cause moderate, systemic complex I inhibition in rats (Betarbet and others 2000). Rotenone, a naturally occurring compound, is a potent inhibitor of complex I, and it is typically used to define the specific activity of the enzyme complex. It is also a commonly used pesticide in vegetable gardens and it is used to

kill nuisance fish in lakes and reservoirs. Rotenone is highly lipophilic and easily crosses the blood-brain barrier, and unlike MPP^+, it does not depend on the dopamine transporter for access to cells.

After administration, rotenone quickly enters the brain (Talpade and others 2000) and causes uniform complex I inhibition throughout the brain (Betarbet and others 2000). Despite this uniform inhibition of complex I, rotenone exposure resulted, over a period of weeks, in highly selective nigrostriatal dopaminergic degeneration, which was virtually identical to that in PD. Additionally, rotenone exposure caused the formation of large cytoplasmic inclusions in nigral neurons. These inclusions contained α-synuclein and ubiquitin, and by electron microscopy were reminiscent of Lewy bodies. Behaviorally, the animals developed bradykinesia, rigidity, and gait problems similar to PD. More severely affected rats had motor "freezing" and flexed posture. Thus, moderate systemic inhibition of complex I was sufficient to reproduce the features of PD (Betarbet and others 2000).

These results have important implications. First, they provide direct experimental evidence that a modest, systemic defect in complex I can cause parkinsonism. Second, they show that substantia nigra dopamine neurons have an intrinsic sensitivity to complex I defects. Despite the fact that complex I was uniformly inhibited throughout the brain, only the substantia nigra neurons degenerated. Third, they give "biological plausibility" to the pesticide/environmental exposure hypothesis of PD.

Complex I and Mechanisms of Neurodegeneration

The mechanisms by which complex I dysfunction leads to selective neurodegeneration in PD remain to be fully elucidated. Nevertheless, it is clear that reduced complex I activity may predispose to excitotoxicity and oxidative damage, both of which have been implicated in PD (Fig. 2).

Complex I dysfunction may render neurons vulnerable to excitotoxic death by altering ATP levels, by impairing Ca^{++} homeostasis, or both. Neurons rely almost exclusively on mitochondrial respiration for production of ATP, and ATP levels play an important role in regulating cellular homeostasis. For example, reduced ATP levels decrease the activity of the plasma membrane Na^+/K^+ ATPase, resulting in partial neuronal depolarization. Neuronal depolarization decreases the voltage-dependent Mg^{++} blockade of the *N*-methyl-D-aspartate (NMDA) glutamate receptor. Under these conditions, even normal levels of extracellular glutamate may cause excitotoxic activation of NMDA receptors and elevation of intracellular Ca^{++}. The magnitude and duration of these Ca^{++} transients may be further elevated by reduced Ca^{++}-ATPase activity due to energy depletion and failure of mitochondrial calcium uptake, both as a result of mitochondrial dysfunction. Moreover, chronic complex I defects have been shown to disrupt normal Ca^{++} signaling in neural cells (Sherer and others 2001b). Thus, complex I impairment may lead to abnormal NMDA receptor activation and, at the same time, render neurons more vulnerable to this insult (Greene and Greenamyre 1996).

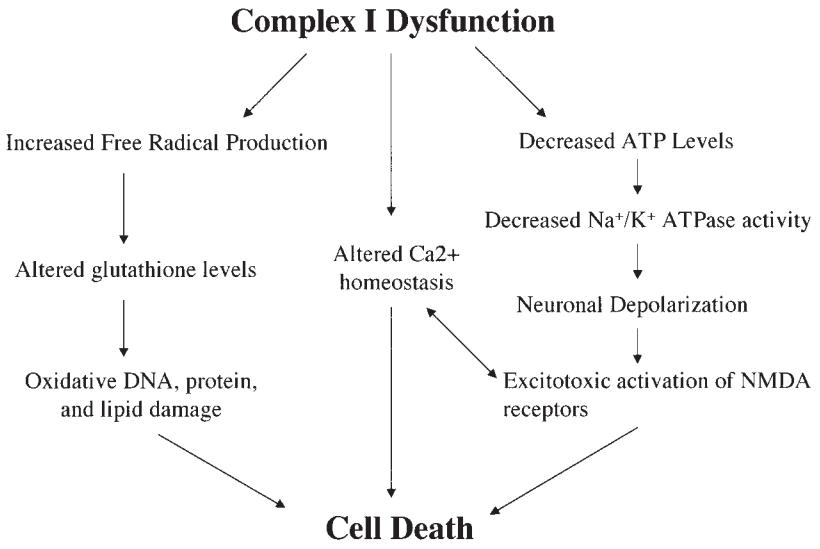

Fig. 2. Mitochondrial dysfunction leads to oxidative stress and renders cells vulnerable to excitotoxic insults. Mitochondrial defects result in increased production of reactive oxygen species (ROS) that consume antioxidants such as glutathione, and cause damage to DNA, protein, and lipids. Complex I dysfunction can also cause altered calcium homeostasis. Additionally, reduced complex I function may cause a drop in ATP production that could decrease the activity of ATPases, such as the Na^+/K^+ ATPase, resulting in neuronal depolarization. Under these conditions, neurons are extremely susceptible to excitotoxic insults.

An increased sensitivity to excitotoxicity may be particularly detrimental in PD. With degeneration of the nigrostriatal dopaminergic nerve terminals, neurons of the subthalamic nucleus become overactive (Bergman and others 1990). These cells are excitatory and glutamatergic, and among their targets is substantia nigra pars compacta. Thus, in the setting of "sick" dopaminergic neurons in PD, there is increased glutamatergic input from the subthalamic nucleus back onto those neurons. It has been suggested that this may lead to excitotoxicity in the substantia nigra (Rodriguez and others 1998). Indeed, we have shown that pharmacological reduction of subthalamic activity reduces chronic degeneration of dopaminergic neurons in a rat model of PD (Blandini and others 2001).

Impairment of complex I may also cause oxidative stress, and there is growing evidence to suggest a role for oxidative damage in PD pathogenesis. Brains from PD patients express elevated markers of oxidative damage including lipid peroxi-

dation and oxidative modifications to proteins and DNA (Dexter and others 1989; Alam and others 1997a, 1997b). The source of this oxidative damage is unknown, but dopamine neurons are believed to exist in a constant state of oxidative stress. The activities of tyrosine hydroxylase, the rate-limiting enzyme of dopamine synthesis, and monoamine oxidase, which catabolizes dopamine, cause the formation of H_2O_2 as a normal by-product. Auto-oxidation of dopamine, which leads to the production of melanin in substantia nigra, also yields H_2O_2, which decomposes to •OH (hydroxyl radical), the most reactive of the reactive oxygen species (ROS). This nonenzymatic reaction is accelerated in the presence of iron (particularly when it is in the free, ferrous form, Fe^{++}), which is abundant physiologically in the substantia nigra. In addition, small amounts of highly reactive free radicals are normally created as a by-product of oxidative phosphorylation, but these are normally handled efficiently by endogenous antioxidant systems. However, partial inhibition of complex I, as produced by rotenone, MPP^+, or observed in PD patients, markedly enhances ROS production (Hensley and others 1998; Hasegawa and others 1990). A dangerous feed-forward cycle may be created in which local ROS production can further damage complex I, causing additional complex I dysfunction and increased ROS production.

Animal models have also pointed toward a role for oxidative stress in PD. Mice exposed to MPTP express elevated levels of ROS and lipid peroxidation (Sriram and others 1997). Antioxidants and spin trap agents reduce MPTP-induced neurodegeneration (Matthews and others 1999). Systemic rotenone-infusion induced selective protein oxidation in the rat striatum (Sherer and others 2001a). In an in vitro system, chronic low-dose rotenone causes progressive depletion of glutathione, oxidative damage to proteins and DNA, release of cytochrome *c* from mitochondria to cytosol, activation of caspase 3, mitochondrial depolarization, and eventually, apoptosis (Betarbet and others 2001).

Table 1. Pesticides Known to Inhibit Complex I

Benzimidazole	Hoe 110779
Bullatacin	Pyridaben
6-Chloro-benzothiadiazole	Pyrimidifen
Cyhalothrin	Sandoz 548A
Fenazaquin	Tebufenpyrad
Fenpyroximate	Thiangazole

From Degli Esposti (1998) and Lümmen (1998).

Table 2. Natural Compounds Known to Inhibit Complex I

Compound	Source
Rotenoids	*Leguminosae* plants
Piericidins*	*Streptomyces* strains
Acetogenins*	*Annonacae* plants (custard apple, paw-paw)
Antibiotics	*Myxobacteria*
Rhein	*Rhubarb*

*More potent than rotenone. From Degli Esposti (1998).

A link also exists between oxidative stress and the formation of cytoplasmic inclusions. Oxidatively modified α-synuclein is more prone to degradation and aggregation than native protein (Souza and others 2000). Additionally, α-synuclein appears to be selectively nitrated in PD (Giasson and others 2000). It is also important to note that the complex I toxins, MPTP (Vila and others 2000) and rotenone (Sherer, Betarbet, and Greenamyre, unpublished results), cause an apparent up-regulation of α-synuclein levels. Together, these results suggest an important role for oxidative stress in PD, and altered complex I function likely contributes to this oxidative burden.

Complex I Inhibitors in the Environment

Studies with rotenone have demonstrated that a mild systemic complex I defect can accurately model PD. Although it is a pesticide, however, rotenone is unlikely to be a major cause of PD. It has poor oral bioavailability and it is reported to undergo rapid biodegradation in the environment. Moreover, it is not used widely in commercial agriculture. In contrast, there are many other pesticides that are known to inhibit complex I (Table 1) and that are used on a much broader scale than rotenone in commercial agriculture (Degli Esposti 1998; Lümmen 1998). Whether these compounds are also capable of causing parkinsonism is unknown and should be investigated.

There is uncertainty about whether PD is a disease of the "industrial age" and whether its incidence is increasing. Nevertheless, it is clear that PD has existed since before there was widespread use of pesticides or other synthetic compounds. Therefore, it is essential to consider natural compounds in our environment, which might be capable of causing PD. In this regard, it is interesting to note that there are many natural substances that potently inhibit complex I (Table 2). Of these, some are substantially more potent than rotenone. It seems likely that there are many other as yet undiscovered compounds that also inhibit complex I.

The extent to which complex I inhibitors, natural or manmade, contaminate our food, water, or air is largely unknown, but this may be a determinant of PD risk. On the other hand, differences in genetic susceptibilities may play a major role in whether or not an individual develops PD. Similarly, there are clear-cut but poorly characterized genetic differences in susceptibility to complex I inhibitors in rodents. For example, strains of mice vary enormously in

their sensitivity to MPTP. In addition, changing from an outbred to an inbred strain of rats greatly reduced the variability in rotenone-induced parkinsonism (Betarbet and others 2000). Thus, there is little doubt that development of PD is a complex interaction of normal aging, genetic susceptibilities, and environment.

Conclusions

The etiology of PD is incompletely understood. Epidemiological studies and experimental models have suggested a role for both environmental exposures and genetic variability in PD pathogenesis. Complex I abnormalities, due to genetic mutations or polymorphisms, or to manmade or natural substances, may play a pivotal role in the pathogenesis of PD by elevating oxidative stress and rendering cells vulnerable to excitotoxic insults (Fig. 3). An improved understanding of the involvement of environmental exposures and mitochondrial defects in PD may ultimately provide a means to prevent PD in susceptible individuals and to slow progression with neuroprotective strategies.

Fig. 3. Involvement of environmental exposures and genetics in Parkinson's disease (PD) pathogenesis. The etiology of PD may result from environmental exposures and/or genetic mutations. Environmental toxins may include pesticides and/or herbicides. Genetic alterations may involve mutations in α-synuclein or parkin genes or alterations in mitochondrial DNA. Additionally, individual genetic variations may impact xenobiotic metabolism. Environmental toxins may directly or indirectly, through influence on genetic factors, lead to PD pathogenesis. Both environmental exposures and genetic mutations may also cause mitochondrial dysfunction resulting in PD.

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