REVIEW

Etiology and Pathogenesis of Parkinson's Disease

Anthony H. Schapira, DSc, MD, FRCP, FMedSci,[1]* and Peter Jenner, PhD, DSc, FRSPharm[2]

[1]*Department of Clinical Neurosciences, Institute of Neurology, University College, London, United Kingdom*
[2]*Neurodegenerative Diseases Research Centre, Institute of Pharmaceutical Sciences, School of Biomedical Sciences, King's College, London, United Kingdom*

ABSTRACT: The past 25 years have seen a major expansion of knowledge concerning the cause of Parkinson's disease provided by an understanding of environmental and genetic factors that underlie the loss of nigral dopaminergic neurons. Based on the actions of toxins, postmortem investigations, and gene defects responsible for familial Parkinson's disease, there is now a general consensus about the mechanisms of cell death that contribute to neuronal loss in Parkinson's disease. Mitochondrial dysfunction, oxidative stress, altered protein handling, and inflammatory change are considered to lead to cell dysfunction and death by apoptosis or autophagy. Ageing is the single most important risk factor for Parkinson's disease, and the biochemical changes that are a consequence of aging amplify these abnormalities in Parkinson's disease brain. What remains to be determined is the combination and sequence of events leading to cell death and whether this is identical in all brain regions where pathology occurs and in all individuals with Parkinson's disease. Focusing on those events that characterize Parkinson's disease, namely, mitochondrial dysfunction and Lewy body formation, may be the key to further advancing the understanding of pathogenesis and to taking these mechanisms forward as a means of defining targets for neuroprotection. © 2011 *Movement* Disorder Society

Key Words: Parkinson's disease; etiology; genetics; environment; pathogenesis; oxidative stress; mitochondria

Determining the cause of Parkinson's disease (PD) has been a focus of neuroscience research for many decades Considerable advances have been made in understanding the environmental and genetic factors that put individuals at risk of developing PD and the molecular mechanisms that underlie neuronal loss. Improved insights into the genetics of PD, enabled through advances in sequencing technology, have perhaps been the driving force to understand the etiology and pathogenesis of PD over the past 15 years.

Initial characterization of the pathology in PD focused attention on the presence of the Lewy body in remaining dopaminergic neurons and the role played by melanin, but only more recently has it been possible to relate these to pathogenic mechanisms, including oxidative stress and altered protein handling.[1] The description of reactive microgliosis in the substantia nigra brought the concept of inflammatory change into a debate over cause and progression of PD.[2] The first description of mitochondrial dysfunction in idiopathic PD[3,4] provided not only a direct link between environmental mitochondrial toxins and PD, but also laid the foundation for what has become the major focus for pathogenesis in genetic PD.[5]

Three specific areas related to the etiology and pathogenesis of PD require particular mention, as these form the basis of this short review. First, the examination of postmortem brain material has uncovered specific components of the cell death cascade, identifying key processes that have subsequently been replicated in experimental models of PD and linked to the events identified in familial forms of PD.[6] Second is the discovery that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) could destroy dopaminergic neurons selectively and thereby generate novel experimental models of PD.[7] Finally is the discovery of

***Correspondence to:** Professor Anthony H. Schapira, Chairman and Head of Department, Department of Clinical Neurosciences, Institute of Neurology, Rowland Hill St., London NW3 2PF, UK; peter.jenner@kcl.ac.uk

Relevant conflicts of interest/financial disclosures: Nothing to report.
Full financial disclosures and author roles may be found in the online version of this article.

Received: 1 March 2011; **Accepted:** 8 March 2011
Published online in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/mds.23732

mutations in α-synuclein in familial PD, which introduced the genetic era from which so many clues to the molecular events occurring as a cause and consequence of cell death have emerged.[8]

The objective of this review is to highlight the milestones that have occurred since the founding of *Movement* Disorders and to put these into the context of what we currently believe to be the pathogenic processes occurring in PD. It is hoped that the reader will perceive not only where studies of the etiology and pathogenesis of PD have taken us but also the limitations of current knowledge, the need to reassess some of what we hold as absolute truth, and finally, where this field needs to move in the next quarter century.

Etiology of Parkinson's Disease

The view of etiological factors in PD has changed remarkably from one of a purely sporadic basis to the view that both environmental and genetic factors contribute to the onset of the illness to a point now where increasingly genetic predisposition must be seen as a major contributor to the underlying cause.[9–11] However, the one factor that most strongly relates to the onset of PD is age or the aging process. Despite the certainty over the role of age, little seems to have been done to understand how age or the aging process is involved.[12] The usual explanation lies in an increased vulnerability of dopaminergic neurons to toxic insult because of increasing failure of normal cellular physiological and biochemical processes. A recent example has been the role of L-type calcium channels as a determinant of the aging of nigral dopaminergic cells.[13] In fact, most concepts of cell death in PD ignore the contribution of age or aging, and in the vast majority of experimental studies, young animals are employed to model the disease process.[14] The same could be said of the predominance of men over women when examining the prevalence of PD.[15] This must also provide a clue to the etiology of the illness, particularly considering the lower life expectancy of men. Despite evidence of the role of estrogen in determining the age of onset of PD in women and the known influence of estrogen on the function of dopaminergic neurons, nothing has emerged to explain the difference.

Environmental influences on the occurrence of PD are known that range from the general, in terms of a potential role for industrialization, rural environment, well water, plant-derived toxins, and bacterial and viral infection, to the specific, as occurs with exposure to organic solvents, carbon monoxide, and carbon disulfide.[16] Recently, there has been increasing interest in pesticide exposure, although there are conflicting outcomes between individual studies, and it is difficult to identify specific pesticide substances that might be responsible for the increase in risk of PD.[17] Specific examples of agrochemicals have been identified that can potentially cause nigral dopaminergic cell death in rodents, namely, paraquat and rotenone.[18,19] Some of these will be discussed later when dealing with pathogenic mechanisms. Factors decreasing the risk of developing PD can also provide valuable clues to its etiology. The evidence for cigarette smoking and caffeine intake in reducing risk appears clear, but there is still uncertainty over the role of others, for example, exercise, anti-inflammatories, antihypertensives (most notably calcium antagonists), and antilipidaemics.[20,21]

Although many specific examples of factors associated with the causation of PD have stimulated interest in the environmental component of the illness, it was the discovery of the neurotoxic effects of MPTP that provided the greatest stimulus and led to a new era of investigation.[22–24] The effects of the discovery of MPTP were far-reaching in that it stimulated studies aimed at relating its mechanism of action to pathogenesis in PD, but perhaps, even more importantly, it provided the first viable model of the motor deficits of PD in primates and a predictive test bed for drug action in humans.[25] However, MPTP-induced parkinsonism differs from PD as it occurs in humans because it is not progressive, Lewy body formation does not occur, and there is no pathology in other areas of the brain affected in the human disease. So, although highly important, MPTP may not convey the critical messages needed to solve the mystery of the etiology of PD.

Postmortem Tissues and Toxins—Clues to Pathogenesis

As indicated earlier, there have been major advances in understanding the mechanisms that underlie nigral dopaminergic cell degeneration from the study of postmortem tissues and toxins that target these cells. The 2 are intertwined, and so the following sections deal with both from the perspective of discrete components of the cell death pathways.

Oxidative stress has long been associated with the death of dopaminergic pathways from the perspective of the production of toxic species through the autoxidation of L-dopa and dopamine and the pathways leading to the formation of neuromelanin.[26,27] The vulnerability of dopaminergic neurons to oxidative stress was highlighted with the discovery of the ability of 6-OHDA to destroy cells following stereotaxic injection into the substantia nigra.[28] More recently, the ability of the redox cycling herbicide. paraquat to destroy nigral dopaminergic neurons after systemic administration has reinforced this view.[29] The detection of increased iron levels in the substantia nigra in PD and alterations in other iron in related parameters provided a mechanism through which free-radical

production might occur, but this is not specific, as it is found in almost all other neurodegenerative diseases.[30] However, deficiencies in the major antioxidant enzyme systems in brain, catalase, superoxide dismutase, and glutathione peroxidase, along with a reduction in the levels of reduced glutathione, suggested that oxidative stress in PD was real and contributing to pathogenesis.[31–33] Subsequently, oxidative damage to lipids, proteins, and DNA was found, confirming that oxidative stress was of functional significance in PD.[34–36] Oxidative stress may not be confined to the brain in PD, as alterations in a variety of markers of oxidative damage are found in the periphery, suggesting a more generalized disease process.[37] What remains unknown is the source and nature of the reactive oxygen species involved. Dopamine metabolism appears an unlikely candidate, as this leads to the formation of hydrogen peroxide rather than the more reactive superoxide or hydroxyl radicals. Also, changes in dopamine metabolism would not explain why some nigral dopaminergic neurons are spared or other dopaminergic areas of the brain are unaffected or how nondopaminergic neurons die in PD.[38]

Mitochondrial Dysfunction in PD

The direct relationship between mitochondrial dysfunction and PD came with the description of complex I deficiency in the substantia nigra of patients who had died with PD[3,4] and was followed by reports of mitochondrial defects in skeletal muscle, platelets, and lymphoblasts in a proportion of cases.[39] The mitochondrial deficiency within the brain appeared to be confined to the nigra.[40] These mitochondrial abnormalities, identified in pathologically confirmed, apparently sporadic PD, were seen against a background of increased oxidative stress and elevated brain iron levels—and emphasized the importance of interconnecting pathways even at this stage of understanding the mitochondrial contribution to PD pathogenesis.[41–46]

The importance of mitochondria to the cause and pathogenesis of PD has been reinforced by the identification of specific mutations of genes that induce dopaminergic cell death and familial PD. Generally, these inherited cases manifest at an earlier mean age than sporadic PD and may include features such as dystonia and cognitive impairment. However, many cases with *PINK1* or *LRRK2* mutations are clinically indistinguishable from sporadic PD. Several expression or knockout models of these mutations have been found to have defects of mitochondrial function.[47]

Parkin is ubiquitously transcribed, and intracellular localization studies have described association of the parkin protein with the endoplasmic reticulum, Golgi apparatus, synaptic vesicles, and mitochondria.[48–50] The function of parkin is not known, but the protein contains a number of different domains for protein–protein interactions and E3 ligase activity. Parkin protein has both a cytosolic and mitochondrial location, depending on the mitochondrial membrane potential (see below). Fibroblasts from *parkin* mutation–positive patients also exhibit decreased complex I activity and complex I–linked ATP production.[51,52]

The PTEN-induced putative kinase 1 (*PINK1*) gene encodes a protein that incorporates a mitochondrial targeting sequence. Mutations in *PINK1* are a cause of autosomal recessive PD.[53] Several homozygous mutations have been described in familial PD patients, both within and outside the kinase domain in autosomal recessive PD. The mitochondrial localization of the PINK1 protein is not affected by the mutations described.

The majority of *PINK1* mutation–positive patients have onset of parkinsonism younger than age 40, most with relatively typical features of PD. Postmortem examination of brains of patients with *PINK1* mutations showed nigrostriatal cell loss and Lewy body formation.[54] Studies have also demonstrated mitochondrial dysfunction in patients with the *PINK1* mutation.[55,56]

Recent studies have demonstrated that PINK1 together with parkin play a vital role in the turnover of mitochondria by autophagy (mitophagy). Parkin translocates from the cytosol to the mitochondrion in response to a fall in mitochondrial membrane potential.[57] Work in PINK1-knockout mouse embryonic fibroblasts and dopaminergic cell lines indicates that parkin and PINK1 involvement in mitophagy includes the ubiquitination of mfn 1 and 2 by parkin.[58] These interactions between parkin and PINK1 explain the data that parkin can rescue biochemical phenotypes from PINK1-knockout models.[59] The importance of the mitophagy pathway in PD is highlighted by the recent demonstration that autophagy appears to be defective in PD nigra and amygdala.[60]

Point mutations or multiplications in the alpha-synuclein gene are a cause of autosomal recessive PD, and alpha-synuclein is a major component of Lewy bodies in idiopathic, apparently sporadic PD. Alpha-synuclein protein is predominantly cytosolic, but a fraction has been identified in mitochondria.[61] Alpha-synuclein binds to membranes and inhibits fusion, leading to fragmentation.[62] This action of alpha-synuclein is independent of the PINK1–parkin mitophagy pathway but can be rescued by overexpression of parkin, PINK1, or DJ1, but not the mutated forms of these genes. Alpha-synuclein has been reported to inhibit complex I in a dose-dependent manner.[63,64] Mitochondrial abnormalities of structure and function have been observed in transgenic mice overexpressing mutant alpha-synuclein.[65] Alpha-synuclein undergoes an important posttranslational modification with

phosphorylation at serine 129,[66] and it would be interesting to determine whether this might influence mitochondrial function.

Lewy Bodies and Microgliosis

The other approach to studying pathogenesis in PD has been to examine those components of the disease process that occur from a pathological perspective. The 2 hallmarks of PD are the appearance of Lewy bodies and the presence of a reactive microgliosis that may contribute to disease progression. The Lewy body has always represented the defining feature of PD, but its relevance to the disease process has remained uncertain, and it was variously attributed to being a marker of pathogenesis and a gravestone for dead and dying neurons. A rekindling of interest in Lewy bodies occurred with the discovery of the mutations of α-synuclein in familial PD and the demonstration that Lewy bodies in sporadic disease were highly immunoreactive for wild-type α-synuclein alongside many other proteins in their normal or damaged states.[67] The subsequent findings of mutations in parkin and UCH-L1 and their roles in the ubiquitin-proteasomal system[68,69] led to the opening of an era of studying alterations in protein handling in dopaminergic cells through both proteasomes and lysosomes (see later). Postmortem investigations and the study of toxins have fueled the ongoing debate. There is a reduction in proteasomal enzyme activity in the substantia nigra that is specific for PD and does not occur in other areas of the brain.[70,71] In addition, there are alterations in the expression of proteasomal subunits and in their regulatory caps that lead to the concept of a different organization of the 26S proteasome, both in PD and in the normal aged brain.[72–74] Whether these changes in proteasomal structure are a cause or consequence of nigral cell loss is an open question because similar changes are also found in the brain in MPTP-treated monkeys long after toxin exposure.[75] What causes the decrease in catalytic activity of the proteasome is unknown but led to the investigation of a number of proteasomal inhibitors for their ability to induce dopaminergic cell death. Lactacystin, epoximycin, and PSI were variously found to be toxic to dopaminergic cell lines and primary fetal VM neurons and following stereotaxic injection into the substantia nigra.[76,77] Excitingly, the peripheral administration of PSI/epoximycin was shown to cause a slowly progressive loss of nigral dopaminergic cells in the rat that led to the onset of motor dysfunction and that was accompanied by neuronal loss in nondopaminergic areas of the brain and by the presence of α-synuclein-positive inclusions.[78] This raised expectations that this would provide the most realistic animal model of PD so far, but this has turned out not to be completely true. The model is difficult, but not impossible, to reproduce, and the effects of PSI may not be as dramatic as first reported.[79–82] However, the observations in postmortem tissue remain robust, and transgenic mice with selective proteasomal deletions also appear to show nigral degeneration.[83]

Recently attention has turned to the role of autophagy in neuronal degeneration in PD. There is an increase in the number of autophagic vacuoles (autophagosomes) in the substantia nigra in PD, and Lewy bodies contain autophagy-related proteins.[60,84,85] The same changes have been observed in cell cultures exposed to toxins such as MPP+ and rotenone and in a transgenic model of PD.[86–88] Chaperone-mediated autophagy is decreased, as judged by reduced expression of LAMP2A and hsc70, and the reduction of LAMP2A levels in dopaminergic cell lines leads reduced chaperone-mediated autophagy and decreased metabolism of wild-type α-synuclein.[60] Importantly, neither the proteasome nor macroautophagy is responsible for the degradation of wild-type α-synuclein, but macroautophagy does undertake the breakdown of the mutant A53T form. Because autophagosomes are formed as part of macroautophagy, this suggests it also is altered in PD. In the MPTP-treated mouse, autophagosome accumulation occurs that is preceded by a marked decrease in the number of lysosomes in dopaminergic neurons because of abnormal permeabilization of lysosomal membranes caused by increased formation of ROS in mitochondria.[85] The linkage between MPTP/MPP+-induced cell death and lysosomal function is further shown by the induction of lysosomal biogenesis, resulting in an increase in lysosomes and a decrease in autophagosomes and leading to decreased MPP+ cell death. In addition, both in vitro and in vivo, enhancement of autophagy by rapamycin attenuates MPTP/MPP+-induced dopaminergic neuronal loss. All this suggests that lysosomes are an important component of pathogenesis in PD but are clearly linked to events that occur in mitochondria and lead to oxidative stress.

Finally, there is the role of reactive microgliosis in PD, which leads to inflammatory change. In postmortem studies, alterations occur in a range of cytokines in both the substantia nigra and the CSF.[89,90] Elevated levels of TNF-α may lead to ceramide-dependent alterations in NKκβ activity and apoptotic cell death. The expression of i-NOS is increased, and this can be linked to increased 3-nitrotyrosine immunoreactivity, indicative of a peroxynitirie attack on proteins. So glial cell activation may take microglia from being protective of neurons in their resting state to pathologically active. There is no answer to the question of whether glial cell activation is a primary cause of dopaminergic cell death or a response to neuronal loss.

However, activated microglia have been detected in the brain of MPTP-treated monkeys and drug addicts many years after toxin exposure, suggesting that once initiated, glial cells may play a role in progressing neuronal loss over long periods.[91–93] The role of glial cell activation is also shown by the ability of the inflammogen lipopolysaccharide to induce dopaminergic cell loss both in vitro and in vivo through its actions on microglia. These effects are also associated with altered cytokine release, decreased trophic factor production, induction of i-NOS, and increased 3-NT expression.[94–99]

Conclusions

Major advances have been made in our understanding of the etiology and pathogenesis of PD over the last 25 years. What is of particular interest, and somewhat reassuring, is that the biochemical abnormalities identified in postmortem sporadic PD brain have mapped to the biochemical consequences of the gene mutations identified as causes of familial PD. Thus, there is a convergence of pathways that appear to lead to dopaminergic, and probably nondopaminergic, cell death in PD. Much has yet to be discovered in terms of identifying the causes of PD, and this review has sought to provide a perspective on only certain aspects; others, specifically detailed discussion of the genetic causes of PD, will be covered by partner articles in this issue of the journal.

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